05-22-07

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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: **Eric T. KOOL**	§	Group Art Unit: **1637**
Serial No.: **10/604,400**	§	Examiner: **Teresa E. Strzelecka**
Filed: **July 17, 2003**	§	Atty. Dkt. No.: **12665.0024.NPUS01**
For: **Detection of Chemical Ligation Using Fluorescence Quenching Leaving Groups**	§	Confirmation No.: **1399**

APPEAL BRIEF

Mail Stop Appeal Brief - Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Appellant submits this Appeal Brief under 37 CFR §41.37 to the Board of Patent Appeals and Interferences in response to the Final Office Action mailed October 17, 2006, Advisory Action mailed March 13, 2007, and in view of the Notice of Appeal filed March 19, 2007. This Appeal Brief is filed within two months from the date of the Notice of Appeal in accordance with MPEP 1205.01. No extension fees are believed to be due. The Commissioner is authorized to deduct the filing fee of $250 for small entity relating to the appeal brief from Deposit Account No. 08-3038/12665.0024.NPUS01. Should any additional fees under 37 C.F.R. §§ 1.16 to 1.21 be required for any reason relating to the enclosed materials, the Commissioner is authorized to deduct said fees from the same Deposit Account.

05/23/2007 TBESHAH1 00000020 083038 10604400
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Respectfully submitted,

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NUMBER: **EV 691724657 US**
DATE OF DEPOSIT: May 21, 2007

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Paula S. Linkhart

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I. REAL PARTY IN INTEREST

The real party in interest is The Board of Trustees of the Leland Stanford Junior University of Palo Alto, California, a University of the State of California, the Assignee of record.

II. RELATED APPEALS AND INTERFERENCES

There are no related appeals or interferences known to Appellant that will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

III. STATUS OF CLAIMS

As of the final rejection and advisory action, claims 1-49 are pending in the application, among which claims 1-14 were rejected, claim 15 was objected to, claims 16-39 were withdrawn from consideration, and claims 40-49 were allowed in the Advisory Action dated March 13, 2007.

The rejections/objection of claims 1-15 are hereby being appealed.

IV. STATUS OF AMENDMENTS

No amendments were filed subsequent to the final rejection.

V. SUMMARY OF CLAIMED SUBJECT MATTER

A. Introduction

The claimed invention relates to a composition comprising a fluorophore compound, which compound comprises a fluorophore group and a fluorescence quenching leaving group. Any fluorophore is generally suitable for the claimed invention, such as fluorescein, TAMRA, Cy3, Cy5, Cy5.5, BODIPY fluorophores, ROX, JOE, and Oregon Green. A fluorescence quenching leaving group in the context of the instant application is a group that is both a quencher and a leaving group, that is, upon ligation of the fluorophore compound molecule with another molecule in intermolecular fashion, or with itself in intramolecular fashion, the quenching leaving group is displaced from the fluorophore compound molecule and the fluorophore is no longer quenched. As a result, the leaving group leaves the fluorophore compound molecule and the fluorescence of the remaining molecule increases.

B. Independent Claims

The only pending independent claim that is involved in the appeal is claim 1.

C. Description

In the instant application, the composition of claim 1 comprises a fluorophore compound, which is described as an oligonucleotide containing a fluorophore and a specialized quencher that also acts as a leaving group. *See*, paragraph [0021] of the instant specification as published in U.S. Patent Application Publication No. 2004/0259102 A1 (a copy is presented under Evidence Appendix.) The quencher that also acts as a leaving group is also called a quenching leaving group or a fluorescence quenching leaving group. *See*, paragraphs [0024], [0025], [0033]-[0037], [0042], [0043], [0047], [0048], [0088] and [0089] of the above-mentioned publication. Structures of exemplary quenching leaving groups are shown in FIGS. 2 and 3 of the above-

mentioned publication. Upon ligation of the fluorophore compound molecule with another molecule in intermolecular fashion, or with itself in intramolecular fashion, the quenching leaving group is displaced from the fluorophore compound molecule, and the fluorophore is no longer quenched. The change in fluorescent properties of the molecule can be detected, providing a quantitative and qualitative assay of ligation. *See*, paragraph [0033] of the above-mentioned publication.

Dependent claims 2-4 characterize the composition of claim 1 as comprising a fluorophore compound having an efficiency of quenching of at least 2 fold, at least 100 fold, and at least 1000 fold, respectively, which subject matters are described in paragraph [0045] of the above-mentioned publication.

Dependent claim 5 characterizes that the fluorophore compound comprised in the composition of claim 1 can be an organic compound, an organometallic compound, a nucleic acid, a peptide, a protein, a lipid, or a carbohydrate as described in paragraph [0034] of the above-mentioned publication.

Dependent claims 6-8 further characterize that the fluorophore compound comprised in the composition of claim 1 is a nucleic acid, either single stranded or double stranded, which subject matters are described in the Abstract and paragraph [0041] as well as illustrated in FIG. 1 of the above-mentioned publication.

Dependent claims 9 and 10 characterize that when the fluorophore compound is a nucleic acid (*see*, claim 6), the quenching leaving group is attached to either the 5' hydroxyl group of the nucleic acid or a hydroxyl group other than the 5' hydroxyl group of the nucleic acid, which subject matters are described in paragraphs [0041] and [0043] of the above-mentioned publication.

Dependent claim 11 characterizes that when the fluorophore compound is a nucleic acid (*see*, claim 6), the fluorophore group is located 1, 2, or 3 nucleotides away from the quenching leaving group as described in paragraph [0045] of the above-mentioned publication.

Dependent claims 12 and 13 characterize that the fluorophore compound comprised in the composition of claim 1 further comprises a nucleophilic group, which can be a phosphorothioate or a phosphoroselenoate, as described in paragraphs [0035] and [0038] of the above-mentioned publication.

Dependent claim 14 provides exemplary fluorophore groups as described in paragraph [0045] of the above-mentioned publication.

Dependent claim 15 provide exemplary fluorescence quenching leaving groups as described in paragraph [0043] as well as illustrated in FIGS. 2 and 3 of the above-mentioned publication.

VI. GROUNDS OF REJECTION/OBJECTION TO BE REVIEWED ON APPEAL

The grounds of rejection/objection to be reviewed on appeal are as follows:

(a) Whether claims 1-8, 10, 11 and 14 are unpatentable under 35 U.S.C. §102(b) over Livak *et al.* (*PCR Meth. Appl.*, vol. 14, pp. 357-362, 1995, "**Livak**", a copy is presented under Evidence Appendix).

(b) Whether claims 1, 5-7, 9 and 12-14 are unpatentable under 35 USC §102(b) over Xu *et al.* (*Nat. Biotechnol.*, vol. 19, pp. 148-152, 2001, "**Xu**", a copy is presented under Evidence Appendix).

(c) Whether claim 15 is unpatentable due to dependency upon rejected claim 1.

VII. ARGUMENT

A. Claim Interpretation

1. The term "fluorophore compound"

In the Office Action dated April 18, 2006, Final Office Action dated October 17, 2006 and Advisory Action dated March 13, 2007, the Examiner states that the term "fluorophore compound" was not defined and that it is interpreted as either a unimolecular or a multimolecular entity. Appellant respectfully disagrees and presents the following arguments.

The instant specification describes fluorophore compounds as oligonucleotides containing a fluorophore and a specialized quencher that also acts as a leaving group (*see*, paragraph [0021] of the above-mentioned publication), which description clearly suggests that the claimed fluorophore compound is a unimolecular entity as an oligonucleotide is a single molecule. In addition, paragraph [0034] of the above-mentioned publication also describes "the molecule containing the fluorophore and the quenching leaving group can be any type of molecule. For example, the molecule can be an organic compound, an organometallic compound, a nucleic acid, a peptide, a protein, a lipid, a carbohydrate, or other types of molecules." (Emphasis added.) The singular form of the exemplary molecule clearly implies that the claimed fluorophore compound is a unimolecular entity. Furthermore, FIGS. 2 and 3 of the instant specification provide examples of the fluorophore compound, which clearly demonstrate the relative locations of the fluorophore group and the fluorescence quenching leaving group on the same molecule as one entity.

Appellant contends that the above descriptions and drawings from the instant specification define the term "fluorophore compound" through the term usage in the context of the specification although no explicit definition is provided therein. In fact, the Court of Appeals

for the Federal Circuit has ruled that the specification is the single best guide to the meaning of a disputed term and that even when guidance is not provided in explicit definitional format, the specification may define claim terms by implication such that the meaning may be found in or ascertained by a reading of the patent documents. See *Phillips v. AWH Corp.*, 415 F.3d 1303, 75 USPQ2d 1321 (Fed. Cir. 2005) (*en banc*); *Vitronics Corp. v. Conceptronic Inc.*, 90 F.3d 1576, 39 USPQ2d 1573, 1577 (Fed. Cir. 1996); and *Novartis Pharms. Corp. v. Abbott Labs.*, 375 F.3d 1328, 1334-35 (Fed. Cir. 2004) (copies are presented under Related Proceedings Appendix.)

In the Final Office Action and/or the Advisory Action, the Examiner argues that the description of the fluorophore compound being "any molecule" does not imply that it is a single molecule. The Examiner further argues that there is no definition of the term, in the sense that there is no statement in the specification of the following form: "The term "fluorophore compound" means...", "The term "fluorophore compound" is..." or "The term "fluorophore compound" refers to...". As such, the Examiner asserts that the interpretation of the term "flurorophore compound" as a unimolecular or multimolecular entity is the broadest reasonable interpretation in view of lack of its definition.

Appellant cannot agree with the Examiner's above reasoning and assertion for two reasons: (1) the above passage of the instant specification (i.e., paragraph [0034]) further provides examples of the type of molecule that the fluorophore compound can be, such as "an organic compound, an organometallic compound, a nucleic acid, a peptide, a protein, a lipid, a carbohydrate" (emphasis added). The singular form of the exemplary molecule clearly implies that the molecule is a single molecule; and (2) as discussed above, the Court of Appeals for the Federal Circuit has ruled that the specification may define claim terms by implication even when there are no explicit definitions. In line with this ruling, Appellant should not have to provide the

claim term meaning in explicit definitional format in order to properly define the claim term. That is, Appellant should not have to provide definitions of the term in those specific forms as asserted by the Examiner in order to properly define the claim term. As such, the Examiner's interpretation of the fluorophore compound as either a unimolecular or a multimolecular entity clearly contradicts the above ruling of the Court of Appeals for the Federal Circuit as the Examiner's so-called "broadest reasonable interpretation" only focuses on lack of explicit definition and ignores the implication provided by the instant specification that the claimed fluorophore compound is a unimolecular entity. Such implication is found in or ascertained by a reading of the instant specification as discussed above.

In view of the above arguments, Appellant respectfully requests that the term "fluorophore compound" in the context of the instant application be interpreted as a unimolecular entity.

2. *The terms "fluorescence quenching leaving group", "leaving group" and "quenching"*

In the Final Office Action and/or the Advisory Action, the Examiner argues that the term "fluorescence quenching leaving group" was not defined and therefore, it is interpreted as any fluorescence quenching group. The Examiner also argues that the terms "leaving group" and "quenching" are described in the specification in terms of their functions and that there are no definitions in the specification. As such, the Examiner asserts that any fluorescent molecule is a leaving group and any fluorescent acceptor molecule is a quencher according to the context of the instant application. Appellant respectfully disagrees and presents the following arguments.

The instant specification clearly sets the metes and bounds of the term "fluorescence quenching leaving group" in that not all fluorescence quenching groups are considered as

fluorescence quenching leaving groups; not all fluorescent molecules are considered as leaving groups; and not all fluorescent acceptor molecules are considered as quenchers according to the context of the instant application.

In particular, the instant specification provides the following definition to the term "leaving groups" in paragraph [0037] of the above-mentioned publication:

> Leaving groups as in general are defined by (a) their ability to activate an atom (to which they are attached) for attack by a nucleophile group and (b) to leave (either simultaneously or subsequently) when the nucleophile does attack.

As to the term "quenching", the instant specification describes that nucleophilic attack on the quenched DNA causes release of the quencher group, which results in a ligated molecule that is now fluorescent due to the absence of the quencher group (*see*, paragraph [0021] of the above-mentioned publication). The instant specification also describes that upon ligation of the fluorophore compound molecule with another molecule in intermolecular fashion, or with itself in intramolecular fashion, the quenching leaving group is displaced from the fluorophore compound molecule and the fluorophore is no longer quenched (*see*, paragraph [0033] of the above-mentioned publication). Furthermore, working examples are provided regarding quenched fluorescence. In particular, Example 2 of the instant specification describes that when beads containing a 7mer MUT probe autoligate a 13mer quenched electrophile probe to themselves, in the presence of the correct target DNA, the beads would become fluorescent, as the dabsylate group was lost and the nearby fluorescein label lost quenching. Such descriptions and examples imply that quenched fluorescence in the context of the instant application involves a quenched electrophile probe, which is a unimolecular entity with both a fluorophore group (e.g., fluorescein) and a fluorescence quenching leaving group (e.g., dabsylate group) located close to

each other thereon. Additionally, multiple examples of fluorescence quencher are also provided (*see*, paragraphs [0043] and [0044] of the above-mentioned publication.)

That is, the above descriptions and examples clearly imply that the fluorescence quenching leaving group in the context of the instant application is not any fluorescence quenching group; rather, it is a group that is both a quencher and a leaving group as a single entity. Appellant contends that the above descriptions and examples define the term "fluorescence quenching leaving group" through the term usage in the context of the specification. As discussed above, the Court of Appears for the Federal Circuit has ruled that the specification may define claim terms by implication even when there are no explicit definitions therein.

Lastly, Appellant wishes to point out that not all fluorescent molecules are in general (not just in the context of the instant application) leaving groups, which is contrary to the Examiner's allegation that basically any fluorescent molecules are inherently leaving groups. In fact, labeled nucleic acids are generally not leaving groups, although they are fluorescent molecules.

For example, the reference of **Livak**, which is cited by the Examiner for one of the art rejections, clearly teaches that the quencher dye (labeled as "Q") remains on the probe even after the cleavage. That is, **Livak**'s fluorescent molecule "Q" is not a leaving group. In addition, Appellant herewith presents Moran et al., *Chem Commun.*, pp. 5003-5005, 2006 ("**Moran**") and Bichenkova *et al.*, *Org. Biomol. Chem.*, vol. 5, pp. 1039-1051, 2007 ("**Bichenkova**") under Evidence Appendix. Both **Moran** and **Bichenkova** references describe nucleic acid detection using fluorescence labeling but no leaving groups.

In view of the above arguments, Appellant respectfully requests that the term "fluorescence quenching leaving group" in the context of the instant application be interpreted as a single entity acting as a quencher and a leaving group simultaneously.

B. Rejection under 35 U.S.C. §102(b) over Livak

The final rejection indicates that claims 1-8, 10, 11 and 14 are unpatentable as being anticipated by **Livak**. In particular, the Examiner alleges that **Livak** teaches the composition of the instant claims.

1. *Claims 1-8, 10, 11 and 14*

Appellant considers claims 1-8, 10, 11 and 14 on appeal stand or fall together. As such, claims 1-8, 10, 11 and 14 will be argued together as a group.

2. *Livak does not Describe Instantly Claimed Composition*

Claim 1 and its dependent claims 2-8, 10, 11 and 14 are directed to a composition of a fluorophore compound, which comprises a fluorophore group and a fluorescence quenching leaving group. Based on the above-discussed interpretations of the terms "fluorophore compound" and "fluorescence quenching leaving group", the instant claims relate to a unimolecular entity which comprises a fluorophore group and a fluorescence quenching leaving group (i.e. quencher/leaving group).

Livak discloses a quenched probe of an oligonucleotide with fluorescent dyes at opposite ends. In particular, the fluorescent dyes are a fluorescein reporter dye (e.g. 6-carboxyfluorescein (6-FAM) phosphoramidite) and a rhodamine quencher dye (e.g. 6-carboxytetramethylrhodamine succinimidyl ester (TAMRA NHS ester)). **Livak** does not teach a fluorescence quenching

leaving group, let alone a fluorophore compound comprising a fluorophore group and a fluorescence quenching leaving group, as claimed in the instant application.

In detail, the rhodamine quencher dye taught by **Livak** is not a "leaving group" as defined in the claimed invention (*see*, paragraph [0037] of the above-mentioned publication for the definition.) First, the rhodamine quencher dye of **Livak** does not activate the atom (to which it is attached) for attack by a nucleophile group. Secondly, it is clear from Figure 1 on page 358 that the quencher dye of **Livak** does not leave the oligonucleotide probe; rather, the fluorescein reporter dye leaves the probe when cleavage occurs between the fluorescein and rhodamine dyes. When the cleavage occurs, the fluorescence intensity of the fluorescein dye increases because the fluorescein is no longer quenched. Also *see* the middle column, page 357 of **Livak**.

Appellant wishes to point out that during the examination, the Examiner has never provided any reasoning as to how the structure of **Livak**'s probe is such that the quencher would leave the probe under nucleophilic attack except a simple statement. In fact, the Examiner's rejection is solely based on her interpretation of the term "fluorescence quenching leaving group" that any fluorescent molecule is a leaving group and any fluorescence quenching group is a fluorescence quenching leaving group.

As discussed above, the Examiner's interpretation of the term "fluorescence quenching leaving group" contradicts the ruling of the Court of Appeals for the Federal Circuit as it only focuses on lack of explicit definitions and ignores implication from the instant specification. Additionally, the Examiner's interpretation of the term and statement that the structure of **Livak**'s probe is such that the quencher would leave the probe under nucleophilic attack is clearly not supported by **Livak**'s teaching. In particular, **Livak** demonstrates that the quencher dye (labeled as "Q") remains on the probe even after the cleavage (especially *see* Figure 1 of

Livak). On the other hand, **Livak** teaches that the fluorescein reporter dye (labeled as "R") leaves the probe after the cleavage. That is, **Livak**'s quencher is not a leaving group. As such, **Livak** does not teach a fluorescence quenching leaving group as claimed in the instant application.

In view of the above remarks, the oligonucleotide probe taught by **Livak** is not the same as the fluorophore compound of the instant application. That is, **Livak** does not describe the composition of the instant claims. As such, the Examiner's rejection of claims 1-8, 10, 11 and 14 under 35 U.S.C. § 102(b) over **Livak** should be reversed.

C. Rejection under 35 U.S.C. §102(b) over Xu

The final rejection indicates that claims 1, 5-7, 9 and 12-14 are unpatentable as anticipated by **Xu**. In particular, the Examiner alleges that **Xu** teaches the composition of the instant claims.

1. *Claims 1, 5-7, 9 and 12-14*

Appellant considers claims 1, 5-7, 9 and 12-14 on appeal stand or fall together. As such, claims 1, 5-7, 9 and 12-14 will be argued together as a group.

2. *Xu does not Describe Instantly Claimed Composition*

Claim 1 and its dependent claims 5-7, 9 and 12-14 are directed to a composition of a fluorophore compound, which comprises a fluorophore group and a fluorescence quenching leaving group. Based on the above-discussed interpretations of the terms "fluorophore compound" and "fluorescence quenching leaving group", the instant claims relate to a unimolecular entity which comprises a fluorophore group and a fuorescence quenching leaving group (i.e. quencher/leaving group).

Xu teaches two 7-mer probes (mutant and wild type) constructed with 3'-end phosphorothioate groups to act as nucleophiles in the ligation reaction and labeled with either the rhodamine (ROX) (for the mutant probe) or hexachlorofluorescein (HEX) (for the wild type probe), as well as one 13-mer probe (universal probe) internally labeled with 5-carboxyfluorescein (FAM). *See*, Sections titled "Design of autoligation probes" on page 148 and titled "Energy transfer probe design" on page 150. **Xu** further teaches that FAM is the donor dye of fluorescence resonance energy transfer (FRET) and ROX or HEX is the acceptor dye of FRET. However, **Xu** does not teach or suggest a fluorescence quenching leaving group for their 13-mer or 7-mer probe, let alone a fluorophore compound as a single molecule comprising a fluorophore group and a fluorescence quenching leaving group, as claimed in the instant application.

In detail, FAM comprised in **Xu**'s 13-mer probe is a fluorophore group that is considered to be the donor dye of FRET. **Xu** is completely silent on any fluorescence quenching leaving group as part of their 13-mer probe. In fact, it is clear from Figure 3 that **Xu**'s 13-mer probe does not comprise a fluorescence quenching leaving group. Whereas, ROX or HEX comprised in their 7-mer probe, considered as the acceptor dye of FRET, is a fluorescence quencher but not a leaving group. *See*, Figure 3 of **Xu**.

In the Final Office Action, the Examiner states that both ROX and HEX do quench the fluorescence of the donor dye as they are acceptor dyes. Appellant does not dispute such statement; however, the difference between **Xu** and the claimed invention is not whether ROX or HEX quenches the fluorescence of the donor dye; rather, it is whether the quencher is also a leaving group. It is clear from the teachings of **Xu** that neither ROX nor HEX is a leaving group.

Appellant wishes to point out that during the examination, the Examiner has never provided any explanation as to why/how **Xu**'s probes would comprise a quenching leaving group. In fact, the Examiner's rejection is solely based on her interpretation of the term "fluorescence quenching leaving group" that any fluorescent molecule is a leaving group and any fluorescence quenching group is a fluorescence quenching leaving group as well as her interpretation of the term "fluorophore compound" as a unimolecular or multimolecular entity.

As discussed above, the Examiner's interpretations of the terms "fluorescence quenching leaving group" and "fluorophore compound" contradict the ruling of the Court of Appeals for the Federal Circuit as it only focuses on lack of explicit definitions and ignores implication from the instant specification. As implied from the instant specification, the fluorophore compound is a unimolecular entity. That is, the fluorophore group and fluorescence quenching leaving group are comprised in the same molecule as a single entity. Also as implied from the instant specification, not all fluorescent molecules are leaving groups and not all fluorescence quenching groups are fluorescence quenching leaving groups. It is clear that neither the 13-mer nor the 7-mer probe taught by **Xu** would be the same as the fluorophore compound of the instant application for two reasons: (1) the fluorescein (i.e., FAM) and the fluorescence quencher (i.e., ROX or HEX) taught by **Xu** are not comprised in the same probe as a single entity; and (2) the fluorescence quencher ROX or HEX is not also a leaving group.

In view of the above remarks, Appellant submits that the 13-mer and 7-mer probes taught by **Xu** are not the same as the fluorophore compound of the instant claims. As such, the Examiner's rejection of claims 1, 5-7, 9 and 12-14 under 35 U.S.C. §102(a) over **Xu** should be reversed.

D. Objection of Claim 15

In the Advisory Action, the Examiner objects to claim 15 with no explanations. Appellant's representative contacted the Examiner inquiring the objection of claim 15 and was informed that claim 15 was not rejected as it was not involved in any of the maintained rejections, but it was objected to since it depended on rejected claim 1.

As discussed above, the Examiner's rejections of claim 1 (and its dependent claims) over **Livak** or **Xu** should be reversed. As such, the objection of claim 15 for depending on rejected claim 1 should also be reversed.



EXHIBIT A

CLAIMS APPENDIX

1. A composition comprising a fluorophore compound, the fluorophore compound comprising a fluorophore group and a fluorescence quenching leaving group.

2. The composition of claim 1, wherein the fluorophore compound is characterized as having an efficiency of quenching of at least 2 fold.

3. The composition of claim 1, wherein the fluorophore compound is characterized as having an efficiency of quenching of at least 100 fold.

4. The composition of claim 1, wherein the fluorophore compound is characterized as having an efficiency of quenching of at least 1000 fold.

5. The composition of claim 1, wherein the fluorophore compound is an organic compound, an organometallic compound, a nucleic acid, a peptide, a protein, a lipid, or a carbohydrate.

6. The composition of claim 1, wherein the fluorophore compound is a nucleic acid.

7. The composition of claim 6, wherein the nucleic acid is single stranded.

8. The composition of claim 6, wherein the nucleic acid is double stranded.

9. The composition of claim 6, wherein the quenching leaving group is attached to the 5' hydroxyl group of the nucleic acid.

10. The composition of claim 6, wherein the quenching leaving group is attached to a hydroxyl group other than the 5' hydroxyl group of the nucleic acid.

11. The composition of claim 6, wherein the fluorophore group is located 1, 2, or 3 nucleotides away from the quenching leaving group.

12. The composition of claim 1, wherein the fluorophore compound further comprises a nucleophilic group.

13. The composition of claim 12, wherein the nucleophilic group is a phosphorothioate or a phosphoroselenoate.

14. The composition of claim 1, wherein the fluorophore group is fluorescein, TAMRA, Cy3, Cy5, Cy5.5, BODIPY fluorophores, ROX, JOE, or Oregon Green.

15. The composition of claim 1, wherein the fluorescence quenching leaving group is a dabsyl group, a dimapdabsyl group, an azobenzene-sulfonyl group, an amino benzenesulfonyl group, a dialkylamino benzenesulfonyl group, a nitro benzenesulfonyl

group, a fluoro benzenesulfonyl group, a cyano benzenesulfonyl group, an arenesulfonyl group, an arylazo-substituted dabsyl group, or a gold particle conjugated to a sulfonyl leaving group.



EXHIBIT B

EVIDENCE APPENDIX

1. U.S. Patent Application Publication No. 2004/0259102 A1
2. Livak *et al.*, *PCR Meth. Appl.*, vol. 14, pp. 357-362, 1995 ("**Livak**")
3. Xu *et al.*, *Nat. Biotechnol.*, vol. 19, pp. 148-152, February 2001 ("**Xu**")
4. Moran *et al.*, *Chem Commun.*, pp. 5003-5005, 2006 ("**Moran**")
5. Bichenkova *et al.*, *Org. Biomol. Chem.*, vol. 5, pp. 1039-1051, 2007 ("**Bichenkova**")

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Oligonucleotides with Fluorescent Dyes at Opposite Ends Provide a Quenched Probe System Useful for Detecting PCR Product and Nucleic Acid Hybridization

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The 5' nuclease PCR assay detects the accumulation of specific PCR product by hybridization and cleavage of a double-labeled fluorogenic probe during the amplification reaction. The probe is an oligonucleotide with both a reporter fluorescent dye and a quencher dye attached. An increase in reporter fluorescence intensity indicates that the probe has hybridized to the target PCR product and has been cleaved by the 5' → 3' nucleolytic activity of *Taq* DNA polymerase. In this study, probes with the quencher dye attached to an internal nucleotide were compared with probes with the quencher dye attached to the 3'-end nucleotide. In all cases, the reporter dye was attached to the 5' end. All intact probes showed quenching of the reporter fluorescence. In general, probes with the quencher dye attached to the 3'-end nucleotide exhibited a larger signal in the 5' nuclease PCR assay than the internally labeled probes. It is proposed that the larger signal is caused by increased likelihood of cleavage by *Taq* DNA polymerase when the probe is hybridized to a template strand during PCR. Probes with the quencher dye attached to the 3'-end nucleotide also exhibited an increase in reporter fluorescence intensity when hybridized to a complementary strand. Thus, oligonucleotides with reporter and quencher dyes attached at opposite ends can be used as homogeneous hybridization probes.

A homogeneous assay for detecting the accumulation of specific PCR product that uses a double-labeled fluorogenic probe was described by Lee et al.[1] The assay exploits the 5' → 3' nucleolytic activity of *Taq* DNA polymerase[2,3] and is diagramed in Figure 1. The fluorogenic probe consists of an oligonucleotide with a reporter fluorescent dye, such as a fluorescein, attached to the 5' end; and a quencher dye, such as a rhodamine, attached internally. When the fluorescein is excited by irradiation, its fluorescent emission will be quenched if the rhodamine is close enough to be excited through the process of fluorescence energy transfer (FET).[4,5] During PCR, if the probe is hybridized to a template strand, *Taq* DNA polymerase will cleave the probe because of its inherent 5' → 3' nucleolytic activity. If the cleavage occurs between the fluorescein and rhodamine dyes, it causes an increase in fluorescein fluorescence intensity because the fluorescein is no longer quenched. The increase in fluorescein fluorescence intensity indicates that the probe-specific PCR product has been generated. Thus, FET between a reporter dye and a quencher dye is critical to the performance of the probe in the 5' nuclease PCR assay.

Quenching is completely dependent on the physical proximity of the two dyes.[6] Because of this, it has been assumed that the quencher dye must be attached near the 5' end. Surprisingly, we have found that attaching a rhodamine dye at the 3' end of a probe still provides adequate quenching for the probe to perform in the 5' nuclease PCR assay. Furthermore, cleavage of this type of probe is not required to achieve some reduction in quenching. Oligonucleotides with a reporter dye on the 5' end and a quencher dye on the 3' end exhibit a much higher reporter fluorescence when double-stranded as compared with single-stranded. This should make it possible to use this type of double-labeled probe for homogeneous detection of nucleic acid hybridization.

MATERIALS AND METHODS

Oligonucleotides

Table 1 shows the nucleotide sequence of the oligonucleotides used in this study. Linker arm nucleotide (LAN) phosphoramidite was obtained from Glen Research. The standard DNA phosphoramidites, 6-carboxyfluorescein (6-FAM) phosphoramidite, 6-carboxytetramethylrhodamine succinimidyl ester (TAMRA NHS ester), and Phosphalink for attaching a 3'-blocking phosphate, were obtained from Perkin-Elmer, Applied Biosystems Division. Oligonucleotide synthesis was performed using an ABI model 394 DNA synthesizer (Applied Biosystems). Primer and complement oligonucleotides were purified using Oligo Purification Cartridges (Applied Biosystems). Double-labeled probes were synthesized with 6-FAM-labeled phosphoramidite at the 5' end, LAN replacing one of the T's in the sequence, and Phosphalink at the 3' end. Following deprotection and ethanol precipitation, TAMRA NHS ester was coupled to the LAN-containing oligonucleotide in 250

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JUNE Vol. 4, No. 6

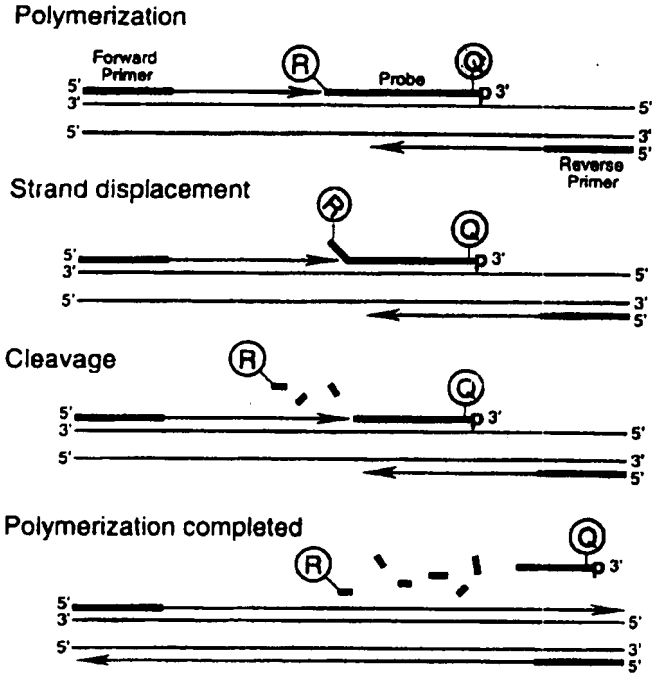


FIGURE 1 Diagram of 5' nuclease assay. Stepwise representation of the 5' → 3' nucleolytic activity of *Taq* DNA polymerase acting on a fluorogenic probe during one extension phase of PCR.

mM Na-bicarbonate buffer (pH 9.0) at room temperature. Unreacted dye was removed by passage over a PD-10 Sephadex column. Finally, the double-labeled probe was purified by preparative high-performance liquid chromatography (HPLC) using an Aquapore C_8 220×4.6-mm column with 7-μm particle size. The column was developed with a 24-min linear gradient of 8–20% acetonitrile in 0.1 M TEAA (triethylamine acetate). Probes are named by designating the sequence from Table 1 and the position of the LAN–TAMRA moiety. For example, probe A1-7 has sequence A1 with LAN–TAMRA at nucleotide position 7 from the 5' end.

PCR Systems

All PCR amplifications were performed in the Perkin-Elmer GeneAmp PCR System 9600 using 50-μl reactions that contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 200 μM dATP, 200 μM dCTP, 200 μM dGTP, 400 μM dUTP, 0.5 unit of AmpErase uracil *N*-glycosylase (Perkin-Elmer), and 1.25 unit of AmpliTaq DNA polymerase (Perkin-Elmer). A 295-bp segment from exon 3 of the human β-actin gene (nucleotides 2141–2435 in the sequence of Nakajima-Iijima et al.)[7] was amplified using primers AFP and ARP (Table 1), which are modified slightly from those of du Breuil et al.[8] Actin amplification reactions contained 4 mM $MgCl_2$, 20 ng of human genomic DNA, 50 nM A1 or A3 probe, and 300 nM each primer. The thermal regimen was 50°C (2 min), 95°C (10 min), 40 cycles of 95°C (20 sec), 60°C (1 min), and hold at 72°C. A 515-bp segment was amplified from a plasmid that consists of a segment of λ DNA (nucleotides 32,220–32,747) inserted in the *SmaI* site of vector pUC119. These reactions contained 3.5 mM $MgCl_2$, 1 ng of plasmid DNA, 50 nM P2 or P5 probe, 200 nM primer F119, and 200 nM primer R119. The thermal regimen was 50°C (2 min), 95°C (10 min), 25 cycles of 95°C (20 sec), 57°C (1 min), and hold at 72°C.

Fluorescence Detection

For each amplification reaction, a 40-μl aliquot of a sample was transferred to an individual well of a white, 96-well microtiter plate (Perkin-Elmer). Fluorescence was measured on the Perkin-Elmer TaqMan LS-50B System, which consists of a luminescence spectrometer with plate reader assembly, a 485-nm excitation filter, and a 515-nm emission filter. Excitation was at 488 nm using a 5-nm slit width. Emission was measured at 518 nm for 6-FAM (the reporter or R value) and 582 nm for TAMRA (the quencher or Q value) using a 10-nm slit width. To determine the increase in reporter emission that is caused by cleavage of the probe during PCR, three normalizations are applied to the raw emission data. First, emission intensity of a buffer blank is subtracted for each wavelength. Second, emission intensity of the reporter is

TABLE 1 Sequences of Oligonucleotides

Name	Type	Sequence
F119	primer	ACCCACAGGAACTGATCACCACTC
R119	primer	ATGTCGCGTTCCGGCTGACGTTCTGC
P2	probe	TCGCATTACTGATCGTTGCCAACCAGTp
P2C	complement	GTACTGGTTGGCAACGATCAGTAATGCGATG
P5	probe	CGGATTTGCTGGTATCTATGACAAGGATp
P5C	complement	TTCATCCTTGTCATAGATACCAGCAAATCCG
AFP	primer	TCACCCACACTGTGCCCATCTACGA
ARP	primer	CAGCGGAACCGCTCATTGCCAATGG
A1	probe	ATGCCCTCCCCCATGCCATCCTGCGTp
A1C	complement	AGACGCAGGATGGCATGGGGGAGGGCATAC
A3	probe	CGCCCTGGACTTCGAGCAAGAGATp
A3C	complement	CCATCTCTTGCTCGAAGTCCAGGGCGAC

For each oligonucleotide used in this study, the nucleic acid sequence is given, written in the 5' → 3' direction. There are three types of oligonucleotides: PCR primer, fluorogenic probe used in the 5' nuclease assay, and complement used to hybridize to the corresponding probe. For the probes, the underlined base indicates a position where LAN with TAMRA attached was substituted for a T. (p) The presence of a 3' phosphate on each probe.

A1-2	RAQGCCCTCCCCCATGCCATCCTGCGTp
A1-7	RATGCCCQCCCCCATGCCATCCTGCGTp
A1-14	RATGCCCTCCCCCAQGCCATCCTGCGTp
A1-19	RATGCCCTCCCCCATGCCAQCCTGCGTp
A1-22	RATGCCCTCCCCCATGCCATCCQGCGTp
A1-26	RATGCCCTCCCCCATGCCATCCTGCGQp

Probe	518 nm		582 nm		RQ⁻	RQ⁺	ΔRQ
	no temp.	+ temp.	no temp.	+ temp.			
A1-2	25.5 ± 2.1	32.7 ± 1.9	38.2 ± 3.0	38.2 ± 2.0	0.67 ± 0.01	0.86 ± 0.06	0.19 ± 0.06
A1-7	53.5 ± 4.3	395.1 ± 21.4	108.5 ± 6.3	110.3 ± 5.3	0.49 ± 0.03	3.58 ± 0.17	3.09 ± 0.18
A1-14	127.0 ± 4.9	403.5 ± 19.1	109.7 ± 5.3	93.1 ± 6.3	1.16 ± 0.02	4.34 ± 0.15	3.18 ± 0.15
A1-19	187.5 ± 17.9	422.7 ± 7.7	70.3 ± 7.4	73.0 ± 2.8	2.67 ± 0.05	5.80 ± 0.15	3.13 ± 0.16
A1-22	224.6 ± 9.4	482.2 ± 43.6	100.0 ± 4.0	96.2 ± 9.6	2.25 ± 0.03	5.02 ± 0.11	2.77 ± 0.12
A1-26	160.2 ± 8.9	454.1 ± 18.4	93.1 ± 5.4	90.7 ± 3.2	1.72 ± 0.02	5.01 ± 0.08	3.29 ± 0.08

FIGURE 2 Results of 5' nuclease assay comparing β-actin probes with TAMRA at different nucleotide positions. As described in Materials and Methods, PCR amplifications containing the indicated probes were performed, and the fluorescence emission was measured at 518 and 582 nm. Reported values are the average±1 S.D. for six reactions run without added template (no temp.) and six reactions run with template (+temp.). The RQ ratio was calculated for each individual reaction and averaged to give the reported RQ^- and RQ^+ values.

divided by the emission intensity of the quencher to give an RQ ratio for each reaction tube. This normalizes for well-to-well variations in probe concentration and fluorescence measurement. Finally, ΔRQ is calculated by subtracting the RQ value of the no-template control (RQ^-) from the RQ value for the complete reaction including template (RQ^+).

RESULTS

A series of probes with increasing distances between the fluorescein reporter and rhodamine quencher were tested to investigate the minimum and maximum spacing that would give an acceptable performance in the 5' nuclease PCR assay. These probes hybridize to a target sequence in the human β-actin gene. Figure 2 shows the results of an experiment in which these probes were included in PCR that amplified a segment of the β-actin gene containing the target sequence. Performance in the 5' nuclease PCR assay is monitored by the magnitude of ΔRQ, which is a measure of the increase in reporter fluorescence caused by PCR amplification of the probe target. Probe A1-2 has a ΔRQ value that is close to zero, indicating that the probe was not cleaved appreciably during the amplification reaction. This suggests that with the quencher dye on the second nucleotide from the 5' end, there is insufficient room for *Taq* polymerase to cleave efficiently between the reporter and quencher. The other five probes exhibited comparable ΔRQ values that are clearly different from zero. Thus, all five probes are being cleaved during PCR amplification resulting in a similar increase in reporter fluorescence. It should be noted that complete digestion of a probe produces a much larger increase in reporter fluorescence than that observed in Figure 2 (data not shown). Thus, even in reactions where amplification occurs, the majority of probe molecules remain uncleaved. It is mainly for this reason that the fluorescence intensity of the quencher dye TAMRA changes little with amplification of the target. This is what allows us to use the 582-nm fluorescence reading as a normalization factor.

The magnitude of RQ^- depends mainly on the quenching efficiency inherent in the specific structure of the probe and the purity of the oligonucleotide. Thus, the larger RQ^- values indicate that probes A1-14, A1-19, A1-22, and A1-26 probably have reduced quenching as compared with A1-7. Still, the degree of quenching is sufficient to detect a highly significant increase in reporter fluorescence when each of these probes is cleaved during PCR.

To further investigate the ability of TAMRA on the 3' end to quench 6-FAM on the 5' end, three additional pairs of probes were tested in the 5' nuclease PCR assay. For each pair, one probe has TAMRA attached to an internal nucleotide and the other has TAMRA attached to the 3' end nucleotide. The results are shown in Table 2. For all three sets, the probe with the 3' quencher exhibits a ΔRQ value that is considerably higher than for the probe with the internal quencher. The RQ^- values suggest that differences in quenching are not as great as those observed with some of the A1 probes. These results demonstrate that a quencher dye on the 3' end of an oligonucleotide can quench efficiently the

TABLE 2 Results of 5' Nuclease Assay Comparing Probes with TAMRA Attached to an Internal or 3'-terminal Nucleotide

Probe	518 nm		582 nm		RQ⁻	RQ⁺	ΔRQ
	no temp.	+ temp.	no temp.	+ temp.			
A3-6	54.6 ± 3.2	84.8 ± 3.7	116.2 ± 6.4	115.6 ± 2.5	0.47 ± 0.02	0.73 ± 0.03	0.26 ± 0.04
A3-24	72.1 ± 2.9	236.5 ± 11.1	84.2 ± 4.0	90.2 ± 3.8	0.86 ± 0.02	2.62 ± 0.05	1.76 ± 0.05
P2-7	82.8 ± 4.4	384.0 ± 34.1	105.1 ± 6.4	120.4 ± 10.2	0.79 ± 0.02	3.19 ± 0.16	2.40 ± 0.16
P2-27	113.4 ± 6.6	555.4 ± 14.1	140.7 ± 8.5	118.7 ± 4.8	0.81 ± 0.01	4.68 ± 0.10	3.88 ± 0.10
PS-10	77.5 ± 6.5	244.4 ± 15.9	86.7 ± 4.3	95.8 ± 6.7	0.89 ± 0.05	2.55 ± 0.06	1.66 ± 0.08
PS-28	64.0 ± 5.2	333.6 ± 12.1	100.6 ± 6.1	94.7 ± 6.3	0.63 ± 0.02	3.53 ± 0.12	2.89 ± 0.13

Reactions containing the indicated probes and calculations were performed as described in Material and Methods and in the legend to Fig. 2.

fluorescence of a reporter dye on the 5' end. The degree of quenching is sufficient for this type of oligonucleotide to be used as a probe in the 5' nuclease PCR assay.

To test the hypothesis that quenching by a 3' TAMRA depends on the flexibility of the oligonucleotide, fluorescence was measured for probes in the single-stranded and double-stranded states. Table 3 reports the fluorescence observed at 518 and 582 nm. The relative degree of quenching is assessed by calculating the RQ ratio. For probes with TAMRA 6–10 nucleotides from the 5' end, there is little difference in the RQ values when comparing single-stranded with double-stranded oligonucleotides. The results for probes with TAMRA at the 3' end are much different. For these probes, hybridization to a complementary strand causes a dramatic increase in RQ. We propose that this loss of quenching is caused by the rigid structure of double-stranded DNA, which prevents the 5' and 3' ends from being in proximity.

When TAMRA is placed toward the 3' end, there is a marked Mg^{2+} effect on quenching. Figure 3 shows a plot of observed RQ values for the A1 series of probes as a function of Mg^{2+} concentration. With TAMRA attached near the 5' end (probe A1-2 or A1-7), the RQ value at 0 mM Mg^{2+} is only slightly higher than RQ at 10 mM Mg^{2+}. For probes A1-19, A1-22, and A1-26, the RQ values at 0 mM Mg^{2+} are very high, indicating a much reduced quenching efficiency. For each of these probes, there is a marked decrease in RQ at 1 mM Mg^{2+} followed by a gradual decline as the Mg^{2+} concentration increases to 10 mM. Probe A1-14 shows an intermediate RQ value at 0 mM Mg^{2+} with a gradual decline at higher Mg^{2+} concentrations. In a low-salt environment with no Mg^{2+} present, a single-stranded oligonucleotide would be expected to adopt an extended conformation because of electrostatic repulsion. The binding of Mg^{2+} ions acts to shield the negative charge of the phosphate backbone so that the oligonucleotide can adopt conformations where the 3' end is close to the 5' end. Therefore, the observed Mg^{2+} effects support the notion that quenching of a 5' reporter dye by TAMRA at or near the 3' end depends on the flexibility of the oligonucleotide.

DISCUSSION

The striking finding of this study is that it seems the rhodamine dye TAMRA, placed at any position in an oligonucleotide, can quench the fluorescent emission of a fluorescein (6-FAM) placed at the 5' end. This implies that a single-stranded, double-labeled oligonucleotide must be able to adopt conformations where the TAMRA is close to the 5' end. It should be noted that the decay of 6-FAM in the excited state requires a certain amount of time. Therefore, what matters for quenching is not the average distance between 6-FAM and TAMRA but, rather, how close TAMRA can get to 6-FAM during the lifetime of the 6-FAM excited state. As long as the decay time of the excited state is relatively long compared with the molecular motions of the oligonucleotide, quenching can occur. Thus, we propose that TAMRA at the 3' end, or any other position, can quench 6-FAM at the 5' end because TAMRA is in proximity to 6-FAM often enough to be able to accept energy transfer from an excited 6-FAM.

Details of the fluorescence measurements remain puzzling. For example, Table 3 shows that hybridization of probes A1-26, A3-24, and PS-28 to their complementary strands not only causes a large increase in 6-FAM fluorescence at 518 nm but also causes a modest increase in TAMRA fluorescence at 582 nm. If TAMRA is being excited by energy transfer from quenched 6-FAM, then loss of quenching attributable to hybridization should cause a decrease in the fluorescence emission of TAMRA. The fact that the fluorescence emission of TAMRA increases indicates that the situation is more complex. For example, we have anecdotal evidence that the bases of the oligonucleotide, especially G, quench the fluorescence of both 6-FAM and TAMRA to some degree. When double-stranded, base-pairing may reduce the ability of the bases to quench. The primary factor causing the quenching of 6-FAM in an intact probe is the TAMRA dye. Evidence for the importance of TAMRA is that 6-FAM fluorescence remains relatively unchanged when probes labeled only with 6-FAM are used in the 5' nuclease PCR assay (data not shown). Secondary effectors of fluorescence, both before and after cleavage of the probe, need to be explored further.

Regardless of the physical mechanism, the relative independence of position and quenching greatly simplifies the design of probes for the 5' nuclease PCR assay. There are three main factors that determine the performance of a double-labeled fluorescent probe in the 5' nuclease PCR assay. The first factor is the degree of quenching observed in the intact probe. This is characterized by the value of RQ^-, which is the ratio of reporter to quencher fluorescent emissions for a no template control PCR. Influences on the value of RQ^- include the particular reporter and quencher

TABLE 3 Comparison of Fluorescence Emissions of Single-stranded and Double-stranded Fluorogenic Probes

	518 nm		582 nm		RQ	
Probe	ss	ds	ss	ds	ss	ds
A1-7	27.75	68.53	61.08	138.18	0.45	0.50
A1-26	43.31	509.38	53.50	93.86	0.81	5.43
A3-6	16.75	62.88	39.33	165.57	0.43	0.38
A3-24	30.05	578.64	67.72	140.25	0.45	3.21
P2-7	35.02	70.13	54.63	121.09	0.64	0.58
P2-27	39.89	320.47	65.10	61.13	0.61	5.25
PS-10	27.34	144.85	61.95	165.54	0.44	0.87
PS-28	33.65	462.29	72.39	104.61	0.46	4.43

(ss) Single-stranded. The fluorescence emissions at 518 or 582 nm for solutions containing a final concentration of 50 nM indicated probe, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, and 10 mM $MgCl_2$. (ds) Double-stranded. The solutions contained, in addition, 100 nM A1C for probes A1-7 and A1-26, 100 nM A3C for probes A3-6 and A3-24, 100 nM P2C for probes P2-7 and P2-27, or 100 nM PSC for probes PS-10 and PS-28. Before the addition of $MgCl_2$, 120 µl of each sample was heated at 95°C for 5 min. Following the addition of 80 µl of 25 mM $MgCl_2$, each sample was allowed to cool to room temperature and the fluorescence emissions were measured. Reported values are the average of three determinations.

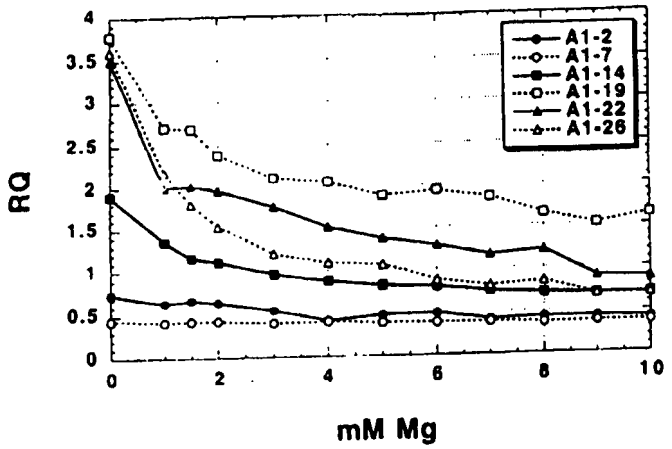


FIGURE 3 Effect of Mg^{2+} concentration on RQ ratio for the A1 series of probes. The fluorescence emission intensity at 518 and 582 nm was measured for solutions containing 50 nM probe, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, and varying amounts (0–10 mM) of $MgCl_2$. The calculated RQ ratios (518 nm intensity divided by 582 nm intensity) are plotted vs. $MgCl_2$ concentration (mM Mg). The key (*upper right*) shows the probes examined.

dyes used, spacing between reporter and quencher dyes, nucleotide sequence context effects, presence of structure or other factors that reduce flexibility of the oligonucleotide, and purity of the probe. The second factor is the efficiency of hybridization, which depends on probe T_m, presence of secondary structure in probe or template, annealing temperature, and other reaction conditions. The third factor is the efficiency at which *Taq* DNA polymerase cleaves the bound probe between the reporter and quencher dyes. This cleavage is dependent on sequence complementarity between probe and template as shown by the observation that mismatches in the segment between reporter and quencher dyes drastically reduce the cleavage of probe.[1]

The rise in RQ^- values for the A1 series of probes seems to indicate that the degree of quenching is reduced somewhat as the quencher is placed toward the 3′ end. The lowest apparent quenching is observed for probe A1-19 (see Fig. 3) rather than for the probe where the TAMRA is at the 3′ end (A1-26). This is understandable, as the conformation of the 3′ end position would be expected to be less restricted than the conformation of an internal position. In effect, a quencher at the 3′ end is freer to adopt conformations close to the 5′ reporter dye than is an internally placed quencher. For the other three sets of probes, the interpretation of RQ^- values is less clear-cut. The A3 probes show the same trend as A1, with the 3′ TAMRA probe having a larger RQ^- than the internal TAMRA probe. For the P2 pair, both probes have about the same RQ^- value. For the P5 probes, the RQ^- for the 3′ probe is less than for the internally labeled probe. Another factor that may explain some of the observed variation is that purity affects the RQ^- value. Although all probes are HPLC purified, a small amount of contamination with unquenched reporter can have a large effect on RQ^-.

Although there may be a modest effect on degree of quenching, the position of the quencher apparently can have a large effect on the efficiency of probe cleavage. The most drastic effect is observed with probe A1-2, where placement of the TAMRA on the second nucleotide reduces the efficiency of cleavage to almost zero. For the A3, P2, and P5 probes, ΔRQ is much greater for the 3′ TAMRA probes as compared with the internal TAMRA probes. This is explained most easily by assuming that probes with TAMRA at the 3′ end are more likely to be cleaved between reporter and quencher than are probes with TAMRA attached internally. For the A1 probes, the cleavage efficiency of probe A1-7 must already be quite high, as ΔRQ does not increase when the quencher is placed closer to the 3′ end. This illustrates the importance of being able to use probes with a quencher on the 3′ end in the 5′ nuclease PCR assay. In this assay, an increase in the intensity of reporter fluorescence is observed only when the probe is cleaved between the reporter and quencher dyes. By placing the reporter and quencher dyes on the opposite ends of an oligonucleotide probe, any cleavage that occurs will be detected. When the quencher is attached to an internal nucleotide, sometimes the probe works well (A1-7) and other times not so well (A3-6). The relatively poor performance of probe A3-6 presumably means the probe is being cleaved 3′ to the quencher rather than between the reporter and quencher. Therefore, the best chance of having a probe that reliably detects accumulation of PCR product in the 5′ nuclease PCR assay is to use a probe with the reporter and quencher dyes on opposite ends.

Placing the quencher dye on the 3′ end may also provide a slight benefit in terms of hybridization efficiency. The presence of a quencher attached to an internal nucleotide might be expected to disrupt base-pairing and reduce the T_m of a probe. In fact, a 2°C–3°C reduction in T_m has been observed for two probes with internally attached TAMRAs.[9] This disruptive effect would be minimized by placing the quencher at the 3′ end. Thus, probes with 3′ quenchers might exhibit slightly higher hybridization efficiencies than probes with internal quenchers.

The combination of increased cleavage and hybridization efficiencies means that probes with 3′ quenchers probably will be more tolerant of mismatches between probe and target as compared with internally labeled probes. This tolerance of mismatches can be advantageous, as when trying to use a single probe to detect PCR-amplified products from samples of different species. Also, it means that cleavage of probe during PCR is less sensitive to alterations in annealing temperature or other reaction conditions. The one application where tolerance of mismatches may be a disadvantage is for allelic discrimination. Lee et al.[1] demonstrated that allele-specific probes were cleaved between reporter and quencher only when hybridized to a perfectly complementary target. This allowed them to distinguish the normal human cystic fibrosis allele from the *ΔF508* mutant. Their probes had TAMRA attached to the seventh nucleotide from

the 5' end and were designed so that any mismatches were between the reporter and quencher. Increasing the distance between reporter and quencher would lessen the disruptive effect of mismatches and allow cleavage of the probe on the incorrect target. Thus, probes with a quencher attached to an internal nucleotide may still be useful for allelic discrimination.

In this study loss of quenching upon hybridization was used to show that quenching by a 3' TAMRA is dependent on the flexibility of a single-stranded oligonucleotide. The increase in reporter fluorescence intensity, though, could also be used to determine whether hybridization has occurred or not. Thus, oligonucleotides with reporter and quencher dyes attached at opposite ends should also be useful as hybridization probes. The ability to detect hybridization in real time means that these probes could be used to measure hybridization kinetics. Also, this type of probe could be used to develop homogeneous hybridization assays for diagnostics or other applications. Bagwell et al.[10] describe just this type of homogeneous assay where hybridization of a probe causes an increase in fluorescence caused by a loss of quenching. However, they utilized a complex probe design that requires adding nucleotides to both ends of the probe sequence to form two imperfect hairpins. The results presented here demonstrate that the simple addition of a reporter dye to one end of an oligonucleotide and a quencher dye to the other end generates a fluorogenic probe that can detect hybridization or PCR amplification.

ACKNOWLEDGMENTS

We acknowledge Lincoln McBride of Perkin-Elmer for his support and encouragement on this project and Mitch Winnik of the University of Toronto for helpful discussions on time-resolved fluorescence.

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Received December 20, 1994; accepted in revised form March 6, 1995.

10/604,400

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Nonenzymatic autoligation in direct three-color detection of RNA and DNA point mutations

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Received 11 September 2000; accepted 10 November 2000

Enzymatic ligation methods are useful in diagnostic detection of DNA sequences. Here we describe the investigation of nonenzymatic phosphorothioate–iodide DNA autoligation chemistry as a method for detection and identification of both RNA and DNA sequences. Combining ligation specificity with the hybridization specificity of the ligated product is shown to yield discrimination of a point mutation as high as >10^4-fold. Unlike enzymatic ligations, this reaction is found to be equally efficient on RNA or DNA templates. The reaction is also shown to exhibit a significant level of self-amplification, with the template acting in catalytic fashion to ligate multiple pairs of probes. A strategy for fluorescence labeling of three autoligating energy transfer (ALET) probes and directly competing them for autoligation on a target sequence is described. The method is tested in several formats, including solution phase, gel, and blot assays. The ALET probe design offers direct RNA detection, combining high sequence specificity with an easily detectable color change by fluorescence resonance energy transfer (FRET).

Keywords: diagnostics, genomics, ligation detection reaction, T4 DNA ligase

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Although oligonucleotides can distinguish single nucleotide differences by small changes in hybridization efficiency, the selectivity seen for oligonucleotide probes of 20 nucleotides or longer is relatively small. As a result, there has been much work carried out recently to find molecular strategies for increasing sensitivity to such small genetic differences[1-16]. One of the most common strategies in current use for engendering high specificity in sequence detection is the use of DNA ligase enzymes[13-24]. Ligases such as the T4 and *Tth* enzymes are quite sensitive to base mismatches at the ligation junction, and selectivities against single mismatches are high, on the order of 10- to 100-fold for T4 DNA ligase[19], and 100- to 10,000-fold for the *Tth* enzyme[21].

Although they are quite useful, enzymatic ligation methods for sequence detection also face some limitations. For example, DNA ligases show low activity with RNAs (ref. 23). In addition, although very short probes exhibit the highest sequence specificity, ligase enzymes are inefficient with oligodeoxynucleotides shorter than about 9 nucleotides[22]. Third, ligase-mediated approaches are unlikely to be useful with modified probes that contain nonnatural DNA backbones such as peptide nucleic acid (PNA), phosphoramidate DNA, or 2′-*O*-methyl RNA. Even relatively simple modifications such as conjugation with biotin or fluorescent labels may be expected to cause difficulties near the ligation junction. Finally, ligase methods are not likely to be useful in intact cellular or tissue preparations, because it would be difficult to deliver the ligase into cells.

As a result, consideration of alternatives to ligase enzymes in the joining of DNA strands seems justified for some applications. A number of nonenzymatic methods that involve the addition of ligating reagents to the DNAs in question have been described[25-28]. Even simpler are ligation chemistries that do not require added reagents; among these are nucleophilic displacements of sulfur on bromoacetyl[29,30], tosyl[31,32], iodo-nucleoside groups[33,34], or photochemically induced ligations[35,36]. Little work has been carried out to investigate the sequence specificity of these methods. In one example, a phosphorothioate–bromoacetyl reaction exhibited selectivities against a mismatch as high as 20-fold in yield[30]. Preliminary work from our laboratory showed that a phosphorothioate–iodide ligation reaction was selective against a mismatch by 180-fold in reaction rate, as judged by use of radiolabeled probes[37].

Here we describe the coupling of competing fluorescent reporter probes in a reagent-free phosphorothioate–iodide ligation reaction in the diagnostic sensing of nucleic acid sequences. This reaction is shown to proceed with very high sequence specificity, and can be used in multiple formats. We show that the reaction can be used in the direct sensing of RNAs or DNAs. A probe set design that allows for three-color discrimination of sequences is described.

Results

Design of autoligation probes. We chose a probe design involving ligation of 7-mer and 13-mer probes (Fig. 1), which can afford very high sequence specificity because of the high mismatch selectivity of the shorter of these probes. Probes were complementary to the H-*ras* protooncogene and to the well-characterized codon 12 G→T mutation[38]. Two 7-mer probes (mutant and wild type) were designed such that the point mutation falls at the center position, to maximize selectivity; these probes were constructed with 3′-end phosphorothioate groups, to act as nucleophiles in the ligation. Coupled with these was a 13-mer probe designed to bind directly adjacent to the mutation probes. This longer "universal" probe is expected to bind equally strongly to wild-type as well as mutant targets. Studies were initially carried out with a single pair of probes at a time (one radiolabeled 7-mer and the 13-mer), and later studies were carried out

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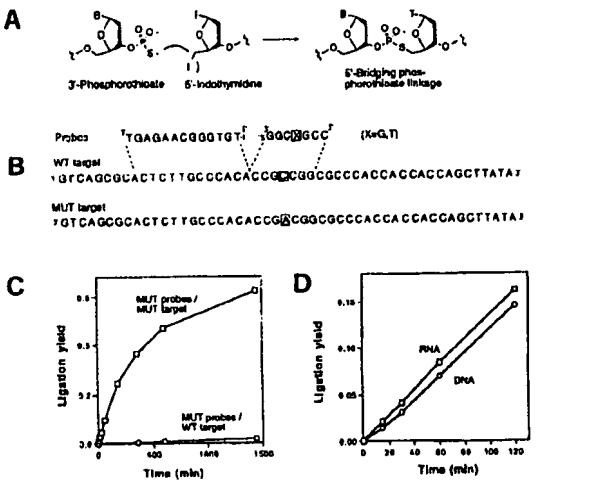


Figure 1. Structures and sequences, and progress of autoligation. (A) Mechanism for autoligation involving 5'-end iodide and 3'-phosphorothioate groups. (B) Probe and target sequences in this study. Iodine end groups are marked with 5'-I, and 3'-end phosphorothioate groups by s. (C) Differing rates of autoligation on matched and singly mismatched target DNA strands (data from ref. 37). (D) Relative autoligation rates on DNA and RNA target strands of identical sequence.

with the three probes simultaneously, each carrying a different fluorescent label (see below).

Course of ligation on DNA and RNA. To investigate the rate of the 7-mer plus 13-mer ligation reaction, and to test whether RNA as well as DNA targets would support the reaction, we prepared synthetic 40-mer targets having the H-*ras* mutant sequence. Ligations were carried out at 25°C at 1 μM probe and target, in a pH 7.0 buffer containing 10 mM Mg^{2+}. Reactions were followed by gel electrophoretic analysis.

Results show that the reaction takes place over a period of hours on the DNA template (Fig. 1C,D), reaching the 50% stage in ~6 h. Significantly, the reaction on the RNA template is observed to occur at virtually the same rate as on the DNA. A single mismatch in the RNA context yielded very little ligated product, with a measured rate that is 180-fold lower than the completely complementary case (data not shown)[39].

To investigate the generality of sequence discrimination, we then constructed a set of four closely related targets (all in the H-*ras* context) with a single variable base (A, T, G, or C), along with 7-mer iodinated probes having the same four bases at the center position. We then carried out kinetics measurements for all 16 possible combinations of probes and targets (Table 1). The most efficiently ligated mismatch in this context is the C-A case, at 4% the rate of the fastest correctly matched ligation. Most mismatches give relative rates of 0.7% or less, compared to the correctly matched cases.

Turnover on a single-stranded template. DNA templates have in some cases been observed to foster the ligation of multiple equivalents of ligating strands, by dissociation of the ligated product followed by binding and ligation of a new pair of ligating strands[27,36,40]. This turnover is commonly inhibited by the stronger binding of the product than the unligated strands to the template, but if it occurred even to a relatively small degree, it would be valuable to possible diagnostic applications, since one target would engender more than one ligated signal. The phosphorothioate–iodide ligation results in a duplex weakly destabilized relative to natural DNA[41], suggesting the possibility of turnover.

To test this, we carried out ligation reactions using 100–10,000 equivalents of probes per equivalent of DNA target strand, and we investigated the yields of ligated product as a function of target concentration, from 1 to 100 nM, and temperature. This was carried out with the same synthetic MUT DNA target as before. Reactions were carried out at 25°C, 37°C, 50°C, or with thermal cycling for 24 h, and were then analyzed by denaturing gel electrophoresis (Table 2). The results show that there is significant turnover of autoligation at the lower target concentrations. The most efficient conversion is seen with the 32°C incubation, under which the binding equilibrium and S_N2 displacement reaction rate may be best balanced. At the lowest target concentration we observe 40 turnovers (producing 280 fmol of ligated signal for 7 fmol of target). In general, cycling is found to increase turnover only by a small amount (by up to 1.5-fold).

Autoligation analysis on double-stranded DNA targets in slot-blot format. To examine whether the autoligation chemistry would proceed on DNAs affixed to nylon membranes, we obtained plasmids encoding wild-type H-*ras* and the codon 12 G→T point mutation. Their sequences were independently confirmed by Sanger sequencing. We prepared two 300-base pair duplexes by PCR, and affixed them to nylon membranes in the slot-blot format. Reactions were carried out with pairs of probes in which the label was present on the shorter MUT probe in 6× saline-sodium citrate (SSC) buffer at 37°C. After ligation, the slot-blot membranes were washed only once for 15 min in 6× SSC to remove any unligated 7-mer. The results were followed by phosphorimaging. Experiments showed that the extent of ligation reached a maximum after ~10 h, and that a strong signal was observed with the complementary mutant DNA (Fig. 2). No signal was observed with radiolabeled 7-mer alone or with probes lacking sulfur (data not shown), confirming that ligation was necessary for producing this signal. Importantly, very little signal was observable under the same conditions with wild-type H-*ras* DNA differing by a single base; quantitation showed a difference of 790-fold in signal for the two closely related targets.

In such a ligation reaction, there are two possible sources of specificity: the ligation reaction itself, and the hybridization selectivity of the ligated product. In the above experiment it was possible that the ligation occurred indiscriminately, and that the complementary ligated 20-mer was remaining bound only to its complementary target. To investigate this possibility we constructed a pre-ligated 20-mer probe and labeled it in identical fashion. It was then hybridized to the two DNAs in the slot-blot apparatus under the same conditions as the ligating probes. Results showed that this 20-mer hybridized almost equally well (1.8:1) to complementary and mismatched targets under these hybridization and low-stringency single-wash conditions, giving equal signals for matched versus mismatched DNA (data not shown). Thus, almost all the selectivity observed above was a result of

Table 1. Survey of autoligation rates for all base-pairing combinations in the context of the H-*ras* system

Base pair (X-Y)[a]	Rate (rel.)[b]	Base pair (X-Y)	Rate (rel.)
T-A	0.89	C-A	0.042
T-C	0.0005	C-C	0.023
T-G	0[c]	C-G	0.45
T-T	0[d]	C-T	0.0046
A-A	0.0068	G-A	0[d]
A-C	0.0070	G-C	1.0
A-G	0[d]	G-G	0.0029
A-T	0.16	G-T	0[d]

[a]In the ligation of d(CCGXCGGs) + d(I-TGTGGGCAAGAGT) in the presence of target strand d(GTCAGCGCACTCTTGCCCACACCGYCGGCGCCCACCAC-CACCAGCTTATA).
[b]Initial rates method; 7–12 data points used for each case. Quantitation by gel electrophoresis and digital fluorescence imaging.
[c]Slope as measured is slightly negative.
[d]Slope measured to be slightly negative but within standard error limits of zero.

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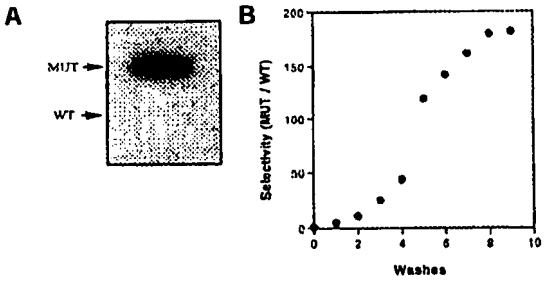


Figure 2. Sequence specificity of autoligation on duplex targets in the slot-blot assay. (A) Comparison of autoligation probe yields on complementary and singly mismatched targets with a single low-stringency wash. (B) Effect of successive SSC washes on increasing specificity of already ligated probes. Total mismatch specificity is the product of autoligation specificity (Table 1) and ligated probe (i.e., product) binding specificity.

the ligation reaction alone. However, under increasing stringency of washing, the ligated 20-mer does shows significant hybridization selectivity (Fig. 2B); after the eighth wash at 60°C with 2× SSC we measured a 180:1 ratio of correct to mismatched signals. The total selectivity achievable by the autoligating probes is the product of the ligation and hybridization selectivities, which here is measured to be between 10^4 and 10^5. Thus, a false positive signal is much lower than the correct signal with equal amounts of DNA, suggesting that such probes could potentially discriminate this mutation in the presence of a 10^4-fold or greater excess of wild-type DNA. Later experiments showed that specificity could be further increased by direct competition of the short probes (see below).

Energy transfer probe design. Fluorescence resonance energy transfer is commonly used to report on the proximity of two molecules or groups[42-45]. We investigated whether FRET could be coupled to the autoligation reaction to give a detectable signal that responds only when the bond is formed. Simple side-by-side binding of two appropriately labeled oligonucleotides on a DNA target is known to result in energy transfer[45]. In the present case we did not wish for such a signal to be observable until ligation occurred, since the reaction adds a high degree of sequence specificity. We felt it possible that the use of one very short probe might prevent simple binding-induced FRET because it might not saturate the binding site adjacent to the longer probe. Thus, our design (Fig. 3) included the use of a 13-mer universal probe internally labeled with the FRET donor

Table 2. Effects of temperature and target DNA concentration on turnover of autoligating probes

Temperature (°C)	Target concentration (nM)	Fold turnover: No cycling[a]	Fold turnover: Cycling[b]
22	1	24	–
	10	1.6	1.0
	100	1.0	1.0
27	1	13	14
	10	1.6	3.0
	100	1.2	1.2
32	1	40	51
	10	4.6	4.7
	100	2.3	2.3
37	1	30	44
	10	5.9	6.2
	100	2.2	2.2

[a]Simple incubation of 10 μM probes with target for 24 h followed by gel electrophoresis and quantitation of ligated product.
[b]24 h of thermal cycling (30 min at temperature shown, followed by 45 s at 95°C).

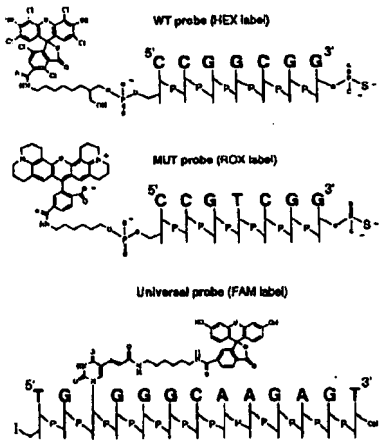


Figure 3. Structures and sequences of fluorescent-labeled autoligating energy transfer (ALET) probes targeted to H-*ras*.

5-carboxyfluorescein (FAM) and a 7-mer mutant probe 5′-end-labeled with the red acceptor dye rhodamine (ROX).

In this dual-probe strategy, one expects to see a color change on detection of H-*ras* codon 12 mutations, but no change in the presence of H-*ras* wild-type DNA. Since such a negative signal might be confused with false negatives (such as when no DNA is present or no ligation occurs), we included a different color change for detection of wild-type DNA. We prepared a third (WT) probe carrying a different FRET acceptor dye, hexachlorofluorescein (HEX) (Fig. 3). If this were ligated to the FAM-labeled universal probe, energy transfer would result in a color change from green to yellow, corresponding to HEX dye emission (556 nm). Simultaneous use of all three probes might be possible, wherein the two short probes carrying acceptor dyes compete for ligation to the universal probe[20].

The three probes were prepared on solid support without the need for post-synthesis modification. In the universal probe, the iodide[34] and FAM–dU conjugate were introduced during standard DNA synthesis. The phosphorothioate group was introduced into the MUT and WT probes by published methods[41] using standard sulfurization chemistry. The HEX dye was incorporated as a commercial phosphoramidite. The ROX dye was added to the terminus of the MUT probe by reaction of an activated ROX NHS ester derivative with a terminal amino group while the DNA remained on the solid support.

Color-based detection of ligation in solution. The three labeled ALET probes were then incubated with 50-nucleotide single-stranded DNAs corresponding to wild-type or codon 12 mutant H-*ras* antisense strand. The reactions were followed by fluorescence spectrometer (Fig. 4). After 1 h of incubation with mutant DNA there was very little spectral change, but after 18 h a significant FRET signal was observed, with quenching of the FAM signal coupled with gain in the ROX signal. An analogous result was observed with the same probe mixture in the presence of wild-type DNA, but with an increase in a yellow HEX signal. No increase in the 602 nm emission is observed in this second experiment, confirming that the WT probe effectively outcompetes the MUT probe for ligation to the universal probe. Although energy transfer and/or ligation appeared to be incomplete in both cases, difference spectra (comparing 1 h to 18 h, Fig. 4C) allow this background to be eliminated, and clearly show the expected energy transfer signals. Significantly, the energy transfer does not occur until ligation brings the two dyes into permanent proximity, since simple hybridization of the probes is expected to be complete within seconds at these concentrations.

The products were also examined by denaturing gel elec-

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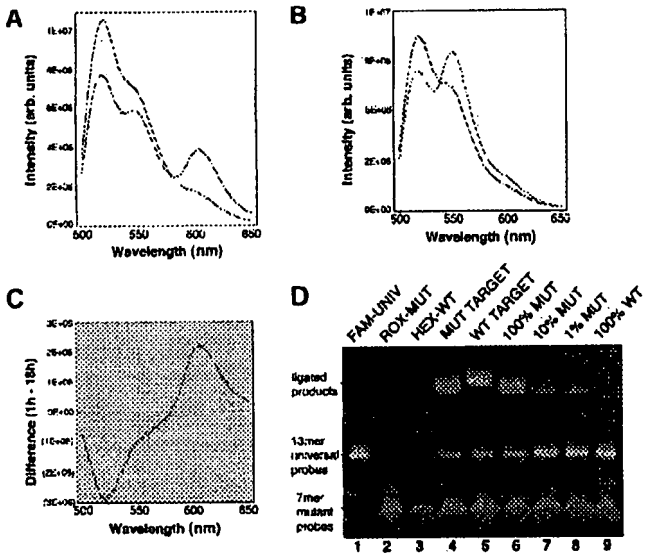


Figure 4. Solution detection and gel-based detection of H-*ras* sequences by ALET probes. (A) Three-probe reaction with MUT DNA target (1h and 18 h). (B) Three-probe reaction with wild-type (WT) target. (C) Difference spectra (1 h–18 h) showing spectral changes characteristic of FRET. Sequences are given in Figure 3. (D) Denaturing polyacrylamide gel showing products of autoligation on MUT and WT target DNAs. All strands are initially at 1.3 μM concentration, and the reactions were incubated 24 h before loading on the gel. The DNAs were imaged by digital camera over a UV transilluminator.

trophoresis (Fig. 4D). In the presence of mutant DNA the ligated probe appears red to the eye, which indicates (1) that energy transfer within the ligated probe is efficient, and (2) that ligation of the mismatched WT (HEX) probe occurs to a very small extent if at all. Conversely, wild-type DNA yields a clearly yellow ligation product having slightly slower mobility, presumably because of the difference in dye structure. None of this retarded yellow band is observable in the mutant DNA ligation. In subsequent ligations the MUT/universal probe pair was used in increasing dilutions with wild-type DNA. The red MUT signal appears to dilute as expected, with no yellow-tagged product visible, suggesting that unintended ligation occurs to a very small extent.

Discussion

The present autoligation chemistry offers a number of potentially useful features. One of these is its ability to be used in the direct detection of RNAs. We are unaware of any other ligation-based genetic detection method in which RNA is detected with equal facility as DNA. These probes can be used with no more difficulty than simple hybridization probes, since no extra reagents or enzymes are needed. Indeed, unreacted probes are more easily washed from targets (such as in blots) because of their very short length. In addition, the ALET probe strategy allows unreacted probes to be easily distinguished from ligated (specifically hybridized) probes because of the change in emission wavelength on ligation. Although multiple dyes are used in this approach, only one excitation source is required (as in ET primers[46]).

With DNA templates the ligation is somewhat slower than enzymatic ligation (by a factor of about two- to fourfold under standard ligation conditions), which increases the assay length. This also limits its utility in real-time PCR sensing, for which alternatives such as Taqman probes[47] and molecular beacons[12] are quite useful. A second notable limitation is that the current reagents allow only for ligations in which the downstream probe has a thymidine at its 5′ end (and thus the target has an adenine at this position). Because there is likely to be at least ±1 nucleotide of latitude in junction placement for a given point mutation, and because the ligation junction can be placed either 5′ or 3′ to the mutation, the restriction is calculated to be a significant hindrance in about 5% of possible sequences.

Ligase enzymes have been used recently in a related FRET strategy[20]. Because it requires no enzyme, the present autoligation reaction offers possible advantages in some applications. For example, ligases, while highly sequence specific, require significantly longer probes than the 7-mers used here. Longer probes would likely remain bound even before ligation, thus rendering the FRET responsive to binding rather than the highly specific ligation. In addition, the use of longer sequences requires higher stringency to wash away unligated probes. Second, ligases have only been able to detect RNAs at high enzyme concentrations, whereas ALET probes show high efficiency and specificity with RNA targets. Third, we have demonstrated that ALET probes can give multiple signals per target, whereas ligase-based methods have not been shown to do so without thermal cycling. Finally, the autoligating probes are simpler to use because they do not need added reagents or enzymes for ligation to occur; it remains to be seen whether this fact will allow autoligations to be useful in intact cells or tissues.

Experimental protocol

Preparation of probes and targets. DNA and RNA oligonucleotides were synthesized on 1 μmol scale on a Perkin Elmer/Applied Biosystems (Foster City, CA) 392 synthesizer using standard β-cyanoethylphosphoramidite coupling chemistry. Phosphorylation was carried out using Phosphate-on reagent from Glen Research (Sterling, VA). For 3′-phosphorothioate sequences, the first nucleotide added after the phosphorylation reagent was sulfurized by the sulfurizing reagent from ABI (Foster City, CA). 5′-Iodo-oligonucleotides were synthesized by published procedures[34,40] using Iodo-T phosphoramidite from Glen Research. Automated RNA synthesis was carried out using *t*-butyldimethylsilyl-protected phosphoramidites from Glen Research. Oligonucleotides were deprotected in 3:1 concentrated ammonia:ethanol at 55°C for 18 h and dried. Silyl protecting groups were removed by adding 400 μl 1 M tetrabutylammonium fluoride in tetrahydrofuran (Sigma, St. Louis, MO) to the residue and shaking at room temperature for 24 h. RNA was desalted on a Sep-pak column (Waters Associates, Milford, MA) and purified by denaturing polyacrylamide gel electrophoresis.

FAM and HEX labels were introduced with FAM–thymidine and 5′-HEX phosphoramidites (Glen Research), during automated DNA synthesis. Labeling of ROX was done by conjugation of the NHS ester with 5′-amino modified DNA. The 5′-amino modifier C6 (Glen Research) was introduced into the 5′ end of the probe during automated DNA synthesis. The monomethoxytrityl (MMT) protecting group was removed by detritylation for 5 min right before the dye labeling reactions. To prevent the potential reaction of the 3′ phosphorothioate with the NHS ester of the dye, the labeling reaction was carried out before DNA was cleaved from the beads. Two milligrams of 6-carboxy-X-rhodamine succinimidyl ester (Molecular Probes, Eugene, OR) were dissolved in 200 μl of dimethylformamide (DMF) immediately before reaction. The reaction solution was prepared by adding 400 μl 0.5 M sodium bicarbonate (pH 7.6) and 400 μl water to the above dye DMF solution. DNA on the silica was added to the mix and allowed to react at room temperature for 1 h with slow shaking (vigorous shaking is to be avoided). DNA beads were recovered by filtering the reaction mixture through glass wool and rinsed with water and acetonitrile several times.

All dye-labeled oligonucleotides were cleaved and deprotected in ammonium hydroxide at room temperature for 24 h, and lyophilized. They were quantitated without further purification using nearest-neighbor methods, subtracting the dye's contribution at 260 nm. The degrees of substitution of all three probes were determined to be above 90%.

Plasmid pT24-C3 (ATCC#41000) containing the c-Ha-*ras* 1 activated oncogene mutation at codon 12 (GGC→GTC) and pbc-N1, containing wild-type c-Ha-*ras* were obtained from American Type Culture Collection (ATCC#41001). Regions of 300 bp including nucleotides –53 (relative to the transcription initiation site) and +244, of normal and activated Ha-*ras*

Detection of a single DNA base-pair mismatch using an anthracene-tagged fluorescent probe†

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Received (in Cambridge, UK) 14th August 2006, Accepted 23rd October 2006
First published as an Advance Article on the web 6th November 2006
DOI: 10.1039/b611650g

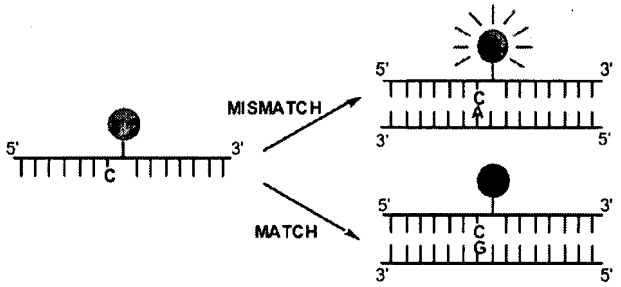
A novel anthracene-tagged oligonucleotide can discriminate between a fully-matched DNA target sequence and one with a single mismatching base-pair through a remarkable difference in fluorescence emission intensity upon duplex formation.

The accurate detection of single-nucleotide polymorphisms (SNPs) in DNA is the subject of intense research. SNPs are thought to be the cause of genetic disorders (most human genetic variations result from SNPs) and thus a fast, reliable and inexpensive sensor is needed for use in DNA diagnostics.[1] Of the many luminescence-based methods used for the detection of mismatches in DNA,[2] one of the simplest is to tag a single fluorophore to an oligonucleotide probe and monitor changes in its emission intensity upon duplex formation. By tagging pyrene to DNA in this manner, particulary promising SNP sensors have been developed.[3]

In contrast to pyrene, only a few examples of anthracene-tagged oligonucleotides have been reported.[4] This is in spite of the well-characterized properties of anthracene, these being its ability to form excimers and undergo [4π + 4π] photocycloaddition, making it a versatile and useful fluorophore.[5] Here we report an anthracene-tagged SNP sensor that can discriminate between fully matched (cognate) and single base-pair mismatched target DNA sequences through quenching and enhancement of fluorescence emission respectively (Scheme 1).

Previous work on pyrene-based SNP sensors[3] has involved tagging the chromophore to a nucleobase. Here a non-nucleosidic unit **5**,[6] containing a serinol linker previously identified as an effective substitute for one nucleoside,[7] was synthesized *via* the route shown[5c] in Scheme 2.†

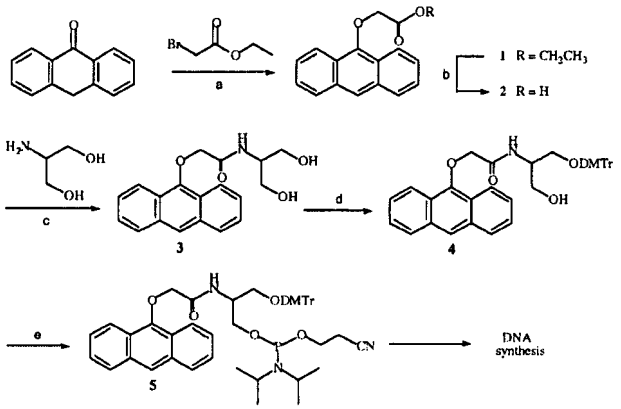
Thus ester **1** was prepared from anthrone and ethyl bromoacetate. Subsequent saponification yielded the corresponding anthracene carboxylic acid **2** following acidification.



Scheme 1 Schematic drawing of the SNP sensing mechanism; a matching CG pair causes quenching whereas a CA mismatch enhances emission.

Carbodiimide-mediated condensation of **2** with serinol afforded the corresponding amide **3** bearing a 1,3-bis alcohol. Using standard reaction protocols, these alcohol functions were sequentially tritylated to the mono-protected racemate **4** and then phosphitylated to afford the novel phosphoramidite **5** as a mixture of diastereomers. This was then inserted at the central position of 15-mer oligonucleotides *via* automated solid-phase synthesis applying extended DNA coupling conditions for the modification.

The two diastereomers (**a** and **b**) of Probe **1** (Table 1) could be separated by RP-HPLC and were thus separately isolated and characterised by MALDI-TOF mass spectrometry (see ESI). The six oligonucleotides synthesised for this study, including a fully



Scheme 2 Reagents and conditions: (a) K_2CO_3, acetone, reflux, o/n, 35%; (b) i. 10% NaOH (aq) soln–EtOH (1 : 1), reflux, o/n; ii. HCl, 93%; (c) HOBt, DIPC, DIEA, DMF, 40 °C, 40 h, 71%; (d) DMTrCl, DMAP, pyridine, rt, o/n, 42%; (e) $(i\text{-}Pr_2N)PClO(CH_2)_2CN$, DIEA, DCM, rt, 1.5 h, 76%.

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† Electronic supplementary information (ESI) available: Experimental procedures for the synthesis and characterisation of anthracene-tagged oligonucleotides and thermal melting curves. See DOI: 10.1039/b611650g

Table 1 Oligonucleotides synthesised in this study

Sequence (X = anthracene tagged unit)	
Probe **1**	5′-TGGACTCXCTCAATG-3′
FM1[a]	5′-CATTGAGAGAGTCCA-3′
1MM[a]	5′-CATTGAGAAAGTCCA-3′
2MM[a]	5′-CATTGAGAATGTCCA-3′
Z[a]	5′-TTAGGGAATTAGCG-3′
FM2[a]	5′-TGGACTCACTCAATG-3′

[a] Purchased from Operon Biotechnologies.

matching sequence, **FM1** and sequences **1MM** and **2MM** with one and two mismatches respectively, are presented in Table 1.

A series of titration experiments were performed on Probe **1** (aqueous phosphate buffer, pH 7) in order to the determine the effect of DNA binding on the anthracene emission signal. As depicted in Fig. 1, the addition of aliquots of the cognate target **FM1** to a 1 μM aqueous solution of Probe **1a**‡ resulted in a significant decrease in anthracene emission intensity, with quenching reaching approximately 85% once one equivalent of target was added. No further decrease was observed in the presence of excess amounts of target, which indicated the formation of a 1 : 1 complex, consistent with DNA duplex formation.

As expected, a titration with a random mis-matching strand **Z** resulted in no significant change in fluorescence emission. However, in contrast to the fully matching system, studies with targets **1MM** and **2MM**, containing one and two mis-matching bases respectively, resulted in an *increase* in emission intensity (Fig. 2). Once again, no additional changes were observed once more than one equivalent of each target was added to the probe.

DNA melting studies† confirmed that duplexes between Probe **1a** and each of the three targets form at the temperature used for the fluorescence studies. The T_m values of these three duplexes, as well as that for **FM1** and its complementary strand§ **FM2**, are presented in Table 2.

In order to explain these results, it is probably significant to note that the stability of the duplex **FM1**–Probe **1a**, where anthracene luminescence is quenched, is very similar to the stability of the duplex **FM1**–**FM2**. It is well documented that anthracene can intercalate into DNA[8] and such an interaction not only increases duplex stability, as evidenced by higher T_m values, but also causes

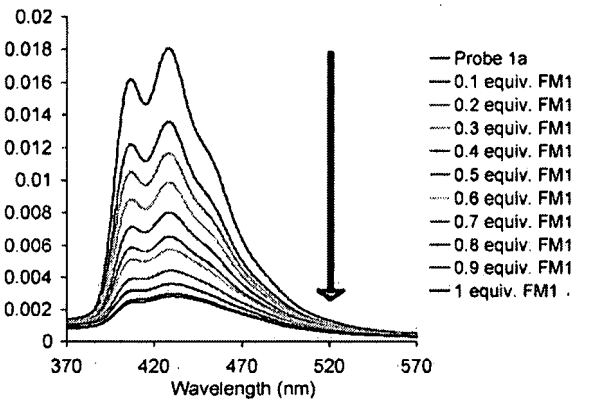


Fig. 1 Overlaid fluorescence spectra for the titration of up to 1 equiv. **FM1** with 1 μM Probe **1a** (0.01 M phosphate, 0.2 M NaCl, pH 7.0 aqueous buffer). λ_{ex} = 350 nm.

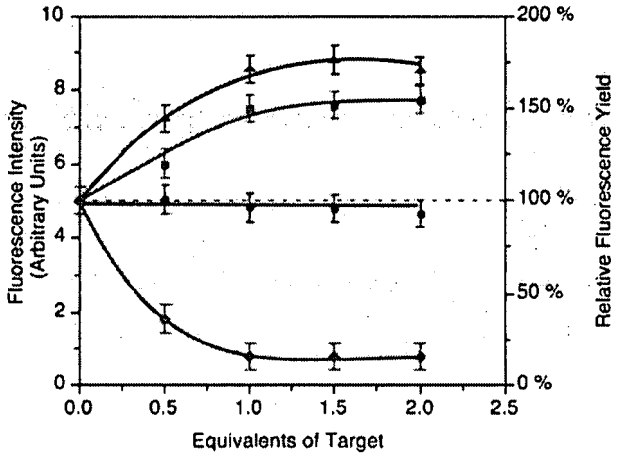


Fig. 2 Fluorescence titration plots at *ca.* 298 K for Probe **1a** with 0.5, 1.0, 1.5 and 2.0 equivalents of targets **FM1** (green), **1MM** (light blue), **2MM** (dark blue) and **Z** (pink) (0.01 M phosphate, 0.2 M NaCl, pH 7.0 aqueous buffer). λ_{ex} = 350 nm.

Table 2 T_M values for duplexes (pH 7.0 buffer, error margin ±1 °C)

Duplex	T_M/°C	Change/°C
FM1–**FM2**	52.0	—
FM1–Probe **1a**	50.0	−2
1MM–Probe **1a**	39.5	−12.5
2MM–Probe **1a**	36.0	−16

luminescence quenching, especially in GC rich regions.[8a,8b] Anthracene intercalation within the **FM1**–Probe **1a** duplex would bring it into stacking contact with at least one GC unit. This would explain the fluorescence changes and also account for there being only a slight change in duplex stability when a stacking adenine base in **FM2** is replaced with a non-nucleosidic linker in Probe **1a**.

On the other hand, it is less clear why duplex formation between Probe **1a** and either **1MM** or **2MM** leads to an increase in emission intensity. Similar behavior has been observed when anthracene intercalates in AT *vs.* GC-rich regions of DNA,[8a] but it is unlikely that the tether in **Probe 1a** is sufficiently long to allow the anthracene to bind distal to its point of attachment. Also, the T_m values clearly indicate that a large decrease in duplex stability results from the introduction of one and two base-pair mismatches, suggesting that for these duplexes, additional stabilisation from anthracene intercalation is not present. Since anthracene is only a weak intercalative DNA binder ($K \approx 150\ M^{-1}$),[8d] it is possible that the presence of a mismatch favours the anthracene being placed in a hydrophobic pocket, which limits quenching by stacking base residues and the aqueous environment.

In conclusion, we have developed a novel anthracene-tagged DNA probe that can detect a single base-pair mismatch in a target sequence which is of relevance to the development of novel SNP sensors. The unusual selectivity of the sensing signal (emission either increasing or decreasing depending on the sequence) stems from the local environment around the anthracene changing in response to duplex formation, rather than it relying on the thermodynamics of duplex formation *per se*.[2a] Studies with different mismatching base sequences using a range of anthracene tags are now planned in order to rationalise these findings further.

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We thank the BBSRC (BMS) for the award of a special studentship (to NM), the EPSRC for the award of a research grant (GR/S07438/02), and the French CNRS and Ministry of Research and Education.

Notes and references

‡ The other diasteroisomer of Probe **1** also appears to be capable of SNP detection. Results will be reported elsewhere in due course.
§ To enable a comparison between the stability of different duplexes formed with **FM1**, the non-nucleosidic base in Probe **1** was changed for a mismatching adenosine in **FM2** as an isosteric replacement.

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Detection of nucleic acids *in situ*: novel oligonucleotide analogues for target-assembled DNA-mounted exciplexes

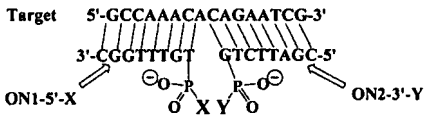
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Received 8th January 2007, Accepted 23rd January 2007
First published as an Advance Article on the web 7th February 2007
DOI: 10.1039/b700293a

This research describes the effects of structural variation and medium effects for the novel split-oligonucleotide (tandem) probe systems for exciplex-based fluorescence detection of DNA. In this approach the detection system is split at a molecular level into signal-silent components, which must be assembled correctly into a specific 3-dimensional structure to ensure close proximity of the exciplex partners and the consequent exciplex fluorescence emission on excitation. The model system consists of two 8-mer oligonucleotides, complementary to adjacent sites of a 16-mer DNA target. Each probe oligonucleotide is equipped with functions able to form an exciplex on correct, contiguous hybridization. This study investigates the influence of a number of structural aspects (*i.e.* chemical structure and composition of exciplex partners, length and structure of linker groups, locations of exciplex partner attachment, as well as effects of media) on the performance of DNA-mounted exciplex systems. The extremely rigorous structural demands for exciplex formation and emission required careful structural design of linkers and partners for exciplex formation, which are here described. Certain organic solvents (especially trifluoroethanol) specifically favour emission of the DNA-mounted exciplexes, probably the net result of the particular duplex structure and specific solvation of the exciplex partners. The exciplexes formed emitted at ~480 nm with large Stokes shifts (~130–140 nm). Comparative studies with pyrene excimer systems were also carried out.

Introduction§

Detection of specific target nucleic acid sequences is commonly achieved by hybridization of a fluorescently labelled oligonucleotide probe to a complementary target. This usually has very large background fluorescence when one compares the same fluorophore-labelled oligonucleotide in its single-stranded and duplex-bound forms.[1] It is possible to decrease the background fluorescence by using two short oligonucleotide probes that are complementary to neighbouring sites of the same DNA target, and can form a pyrene excimer (X = Y = pyrene in Fig. 1). Excitation of one of the pyrene partners, forms an excited state dimer (excimer) that emits at long wavelength (Stokes shifts >100 nm).[2–8] Thus, the target assembles its own detector from components which are non-fluorescent at the detection wavelength, giving a massive improvement in terms of the reduced background.



Identities of X and Y are shown in Table 1.

X=OH giving ON1-5'-phosphate Y=OH giving ON2-3'-phosphate

Fig. 1 Schematic diagram of DNA-mounted exciprobes. ON1-5'-X and ON2-3'-Y represent oligonucleotide split-probes with covalently attached exciplex partners X and Y, respectively. The oligonucleotide parts of the split-probes ON1 and ON2 possess sequences 5'-pdTGTTTGGC and dCGATTCTG-3'p, respectively. GCCAAACACAGAATC is the target DNA. Structural variants of the exciprobes tested, along with nomenclature, are shown in Table 1.

For excimer–DNA systems, X and Y must be identical. More variety in the molecular properties of such systems and hence their potential applications would be possible if X and Y could be different from each other and could be varied (*i.e.* exciplexes),[9,10] which provides a clear advantage for exciplex-based detecting systems over excimer-based analogues. Being able to vary the chemical and physical natures of X and Y should allow exciplex-assembling systems of a range of properties to be constructed increasing their potential for applications, for example, tuning of properties to provide detectors matched to specific needs, or possibly for multiplexing. In model organic intramolecular systems corresponding to those exciplex partners used in this DNA system, the wavelengths of exciplex emission were strongly affected by the identities of X and Y.[11] To achieve efficient exciplex or excimer formation, the partners X and Y must be able to approach

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§ Abbreviations used: BOP, benzotriazol-1-yloxytris(dimethylamino)-phosphonium; I_E/I_M, ratio of exciplex or excimer fluorescence intensity to that of the corresponding locally excited state for the pyrene or perylene component of the system; LES, locally excited state; ON1, oligonucleotide 1 (5'pdTGTTTGGC); ON2, oligonucleotide 2 (dCGATTCTG3'p); ou_{260}, the amount of oligonucleotide that gives an optical density of 1 at 260 nm for a 1 cm pathlength; SNP, single nucleotide polymorphism; λ_{ex}, excitation wavelength maximum; λ_{em}, wavelength emission maximum.

each other very closely (typically the distance is ~4 Å for the pyrene excimer[9,10]). This distance is of the order of the thickness of a base pair. Thus, the potential resolution of the exciplex or excimer systems theoretically approaches a single base pair. In contrast, FRET systems are effective over much greater distances (10–100 Å; >~3 bp) and hence of low resolution.

While excimer-based systems have been successfully applied to nucleic acid detection,[3,5,7,8,12] exciplex-based split-probe systems have been introduced only recently and studied to a much lesser extent.[1,13,14] There are several differences[15] in the fundamental natures of excimers and exciplexes. The most problematic difference in terms of DNA detection is the much greater solvent sensitivity of exciplexes compared to excimers. For solvents of polarity greater than approximately that of acetonitrile intramolecular organic exciplex emission is usually totally quenched,[16–25] although there are some exceptions,[26,27] and very recently we described an organic intramolecular exciplex system that emits even in solvents with a dielectric constant of over 180.[11] Recently Kashida *et al.*[14] reported exciplex emission in aqueous solution from pyrene and *N,N*-dimethylaniline moieties incorporated into the middle of DNA probes with the exciplex partners directed internally and lying in the centre of a (distorted) duplex. This approach allowed successful detection of one-base deletion in DNA sequences, but as it requires formation of specific secondary structures (*e.g.* loops), the unambiguous signalling of single base changes has not yet been reported for it. We have introduced an alternative approach,[1,13] which utilised two separate oligonucleotide split-probes, each equipped with exciplex partners that are oriented towards the bulk medium. We also reported assay conditions[1,13] under which DNA-based exciplexes can be strongly emissive for systems constructed according to the design of Fig. 1, and allow the clean detection of single base mismatches and thus the potential for SNP calling. However, only one initial prototype pair of exciplex partners was described in detail.[13] Thus, we now report the influence of a number of structural aspects (*i.e.* chemical structure and composition of exci-partners, length and structure of linker groups, locations of exci-partner attachment, as well as effects of media) on the performance of DNA-mounted exciplex systems conceptualised as in Fig. 1. The 16-mer-target nucleotide sequence (Fig. 1) was chosen to avoid formation of secondary structure. The exciplex is formed between various donor and acceptor moieties (see Table 1 for structures) covalently attached to the 5′- and 3′-terminal phosphate groups, respectively, of the 8-mer oligonucleotides shown in Fig. 1 (referred to as the probe oligonucleotides or exciprobes). The structures and nomenclature used for the systems studied are shown in Fig. 1 and Table 1.

Results

Split-probe system in aqueous solutions

Split-probe systems SP-1–SP-41 (Table 1) were constructed using appropriate combinations of potential exciplex-forming oligonucleotide-probe components ON1-5′-X and ON2-3′-Y mixed with 16-mer target in 0.01 M Tris buffer (pH 8.3), containing 0.1 M NaCl at 10 °C. In the absence of target, mixing equimolar amounts of two exciprobe components ON1-5′-X and ON2-3′-Y (2.5 μM) did not alter λ_{max} for emission or excitation of the components, indicating no interaction between the probes in the absence of target. Adding the target strand led to strong quenching of the pyrene emission (LES), and λ_{max} for emission shifted from 340 nm to higher wavelength (typically by 3–7 nm). This evidence of hybridisation in the full duplex tandem system was confirmed by the A_{260} melting profiles.[13] However, none of the potential exciplex systems studied showed exciplex emission in such purely aqueous conditions, although excimer emission at ~480 nm was observed for SP-1 under identical conditions (Fig. 2).

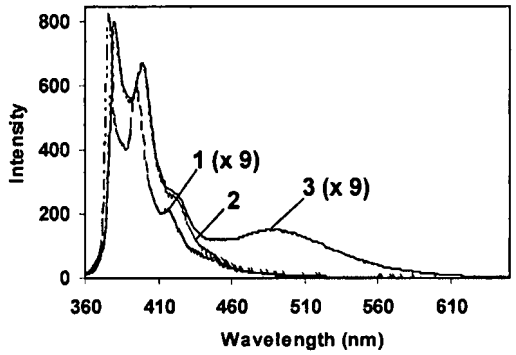


Fig. 2 Fluorescence spectra of the excimer-forming SP-1 system at different stages of self-assembly in pure aqueous solution. **1**, ON1-5′-Pyr probe alone; **2**, ON1-5′-Pyr + ON2-3′-Pyr; **3**, fully assembled SP-1 system (ON1-5′-Pyr + ON2-3′-Pyr + Target). Spectra were recorded at 5 °C in 10 mM Tris buffer, pH 8.3, 0.1 M NaCl (λ_{ex} 345 nm, slitwidth 3). Spectra **1** and **3** were re-scaled to the 380 nm pyrene LES emission band in spectrum **2** using a scaling factor of 9. The intensity of the pyrene (LES) peaks for systems with a 3′-pyrene label is usually very much greater than for similar systems with the pyrene on a 5′-site. For this reason the intensity observed for system **2** (which contains a 3′- as well as a 5′-pyrene-labelled probe, mutually non-interacting) is much greater than for system **1** with only a 5′-pyrene probe present in solution, and it was useful to scale the data.

Influence of co-solvents on exciplex signals from split-probe oligo systems

Exciplex emission was specifically enhanced by trifluoroethanol (TFE).[13] Fig. 3 (curve 1) shows the emission spectrum of the

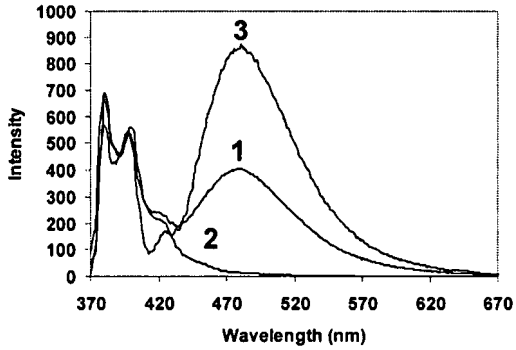


Fig. 3 Fluorescence spectra of the SP-19 split-probe system (**1**) and a control system (ON1-5′-Pyr + ON2 + target) with unmodified ON2 oligo-probe lacking the *N,N′*-dimethylnaphthalene exci-partner (**2**) recorded at 10 °C. Spectrum (**3**) is the emission spectrum of re-annealed SP-19 system following a heating–re-cooling cycle. Spectra were recorded in Tris buffer (10 mM Tris, pH 8.3, 100 mM NaCl) containing 80% TFE v/v (excitation at 350 nm).

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Table 1 Structures and nomenclature of modified oligonucleotides used

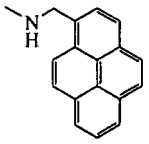
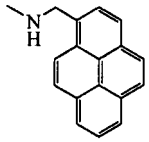
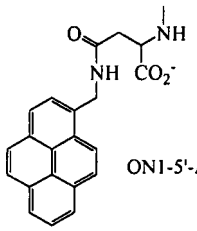
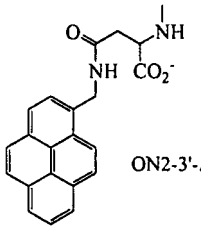
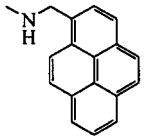
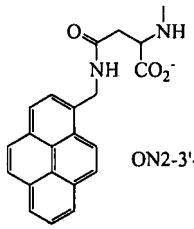
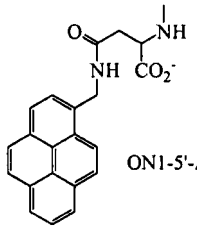
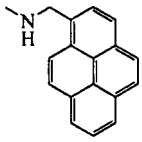
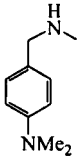
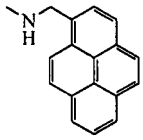
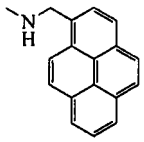
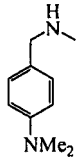
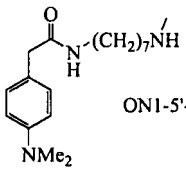
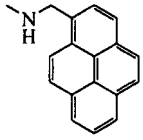
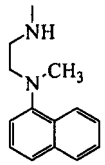
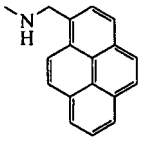
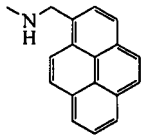
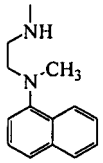
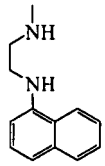
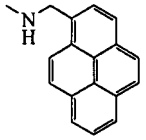
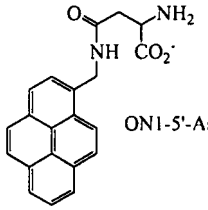
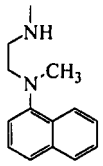
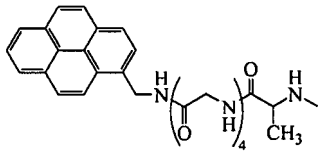
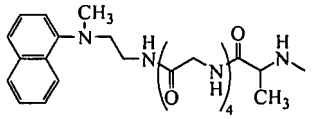
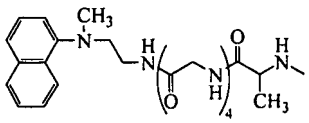
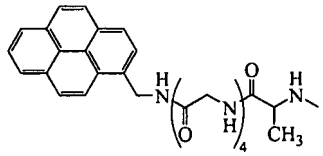
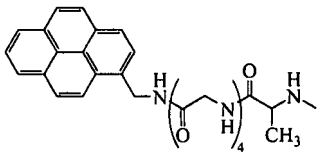
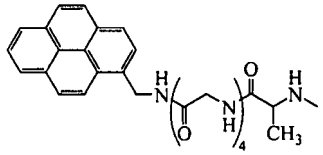
Code	Exci-partner X (to give ON1-5'-X exciprobe)	Exci-partner Y (to give ON2-3'-Y exciprobe)
Excimer systems		
SP-1	 ON1-5'-Pyr	 ON2-3'-Pyr
SP-25	 ON1-5'-AspPyr	 ON2-3'-AspPyr
SP-40	 ON1-5'-Pyr	 ON2-3'-AspPyr
SP-41	 ON1-5'-AspPyr	 ON2-3'-Pyr
Exciplex systems		
SP-2	 ON1-5'-DMA	 ON2-3'-Pyr
SP-4	 ON1-5'-Pyr	 ON2-3'-DMA
SP-3	 ON1-5'-C7DMA	 ON2-3'-Pyr

Table 1 (*Contd.*)

Code	Exci-partner X (to give ON1-5′-X exciprobe)	Exci-partner Y (to give ON2-3′-Y exciprobe)
SP-18	 ON1-5′-NMeNp	 ON2-3′-Pyr
SP-19	 ON1-5′-Pyr	 ON2-3′-NMeNp
SP-20	 ON1-5′-NHNp	 ON2-3′-Pyr
SP-26	 ON1-5′-AspPyr	 ON2-3′-NMeNp
SP-27	 ON1-5′-Pept-Pyr	 ON2-3′-Pept-NMeNp
SP-28	 ON1-5′-Pept-NMeNp	 ON2-3′-Pept-Pyr
SP-29	 ON1-5′-Pept-Pyr	 ON2-3′-Pept-Pyr

SP-19 split-probe exciplex system (ON1-5′Pyr + ON2-3′NMeNp + target strand) recorded at 10° C in Tris buffer containing 80% TFE. As in fully aqueous solutions, on hybridisation of probes to target there was a bathochromic shift and quenching of the pyrene LES emission. The new, broad emission band at ~480 nm (Fig. 3) in the presence of target strand at 80% TFE is attributable to exciplex formation between the pyrenyl and dialkylnaphthyl exci-partners. A control experiment (curve 2) under identical

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conditions, but without an *N,N*-dialkylaminonaphthaleno exci-partner at the 3′-terminal phosphate group of ON2 (leaving a free 3′-phosphate), revealed no exciplex emission at 480 nm, although there was quenching and red shift of the LES emission, indicating hybridisation of the probes with the target. Thus, the 480 nm emission band was attributed to interaction between the two exci-partners, not interaction of the exci-partner(s) with the nucleotide bases. An additional control experiment of mixing the two oligo probes in the absence of the target DNA sequence (data not shown) resulted in no exciplex emission at 480 nm, demonstrating that tandem hybridisation to the target is necessary for the exciplex signal development.

A heating–re-cooling cycle can significantly improve the exciplex signal (Fig. 3, curve 3). Heating the system (up to 70 °C) results in duplex melting and subsequent separation of exciplex partners, which causes the exciplex band to disappear (accompanied by an increase in LES emission intensity and λ_{em} shifting back to that for the ON1-5′Pyr probe). On cooling back to 10 °C the oligonucleotide chains re-anneal and the full tandem system reassembles with concomitant exciplex emission of greater intensity than before the heating–re-cooling cycle. The increased intensity is likely to be the result of thermodynamically achieving a tandem duplex conformation with more favourable orientations of the exci-partners. This phenomenon was observed for various split-probe systems and assigned to improved mutual orientation of exci-partners (in terms of exciplex assembly) as a result of the slow cooling. The heating–cooling cycle presumably disrupts undesirable, competitive hydrophobic interactions of the exci-partners with the DNA, freeing them for exciplex formation.

Fig. 4 shows emission spectra of the split-probe exciplex SP-19 system (ON1-5′-Pyr + ON2-3′-NMeNp + target strand) in Tris buffer containing various percentages of TFE (spectra were recorded without heating–re-cooling optimisation). A plot of the effect of percentage TFE on the ratio (I_E/I_M) for SP-19 is shown in the inset to Fig. 4, where I_M is intensity of fluorescence emitted by the pyrene LES at 376 nm and I_E is intensity of the exciplex band at 480 nm. The plot shows that the strongest exciplex emission

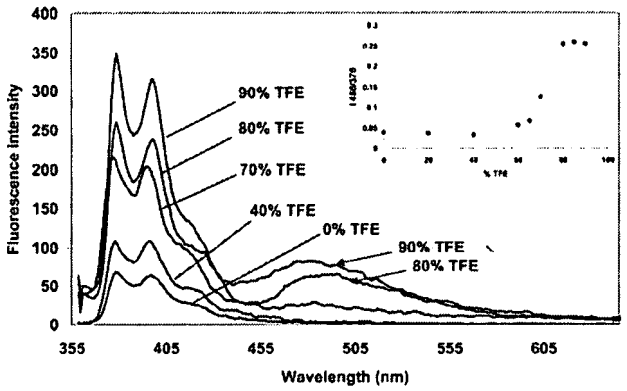


Fig. 4 Effect of percentage trifluoroethanol (v/v as indicated on the spectra labels) on exciplex emission for the SP-19 system. Spectra were recorded at 5 °C with component concentrations of 2.5 μM in 10 mM Tris, pH 8.3, containing 0.1 M NaCl and percentage of TFE as indicated by labels; λ_{ex} 350 nm, slit width 5 nm. Spectra were base-line corrected for small contributions from buffer- and co-solvent background. The inset shows a plot of the ratio of exciplex fluorescence to LES fluorescence (I_E/I_M) *versus* percentage TFE (v/v).

occurs once a level of ≥80% TFE has been reached. In addition, the maximum wavelength of the excitation spectrum is influenced by a higher percentage of TFE: 340 nm for 70%; 350 nm for 80%; 348 for 85% and 335 nm for 90%. Values of λ_{max} for exciplex emission at various percentages of TFE also varied (Fig. 4) and were (percentages of TFE in parentheses): 465 nm (60%), 483 nm (70%), 482 nm (80%), 480 nm (85%) and 481 nm (90%). The wavelength shift of the excitation spectrum may arise from a simple solvent effect on the excitation spectrum of the exciplex and/or from a structural change in the environment of the exciplex partners with respect to each other and the DNA to which they are chemically bound.

Similar observations were made for the pyrene excimer (SP-1 split-probe system) assembly. Excimer fluorescence at 481 nm increased in intensity relative to the LES peak by means of a heating–re-cooling cycle. For example, in 80% TFE/pH 8.3 Tris medium I_E/I_M changed from 2.24 to 2.74 by heating from 10 °C to 70 °C over 5 minutes followed by cooling back to 10 °C over 15 minutes. In addition, excimer emission for SP-1 was also augmented by the presence of TFE: I_E/I_M values were (percentage of TFE in parentheses): 0.24 (20%), 0.46 (40%), 0.62 (60%) and 2.24 (80%).

Effect of reversing relative locations of donor and acceptor partners

Exciprobe performance can be strongly affected by the relative location of donor and acceptor partners within the oligo probe systems, *i.e.* by their relative attachment sites at the 3′- and 5′-juxtaposed ends of the hybridised short probe oligonucleotides, ON1 and ON2 in Fig. 1. Fig. 5 compares the fluorescence emission spectra for systems SP-19 *vs.* SP-18 (**A**, spectra **1** and **2**, respectively) and SP-4 *vs.* SP-2 (**B**, spectra **3** and **4**, respectively) in which the relative locations of the acceptor partner (pyrene) and the donor (*N,N*-dialkylaminonaphthalene (for SP-18 and SP-19, **A**), or *N,N*-dimethylaminobenzyl (for SP-2 and SP-4, **B**) were reversed. Since the formation of the exciplex/excimer band is accompanied by quenching of the LES signal, I_E/I_M (the ratio between the intensity of the LES pyrene emission and the exciplex/excimer emission) is a good measure of formation of excited state complex/dimer efficiency.[28] As a rule, a higher *exciplex:LES* ratio (I_E/I_M) was observed with the pyrenyl group at the 5′-terminal phosphate site of oligo probe ON1 and the electron-donating group (*i.e.* *N,N*-dialkylaminonaphthalene for SP-19 or *N,N*-dimethylaminobenzyl for SP-4) at the 3′-terminal phosphate site of oligo probe ON2 (spectra 1 and 3, respectively). Changing the relative (3′- or 5′-) locations of the electron donor and acceptor partners to give systems SP-18 and SP-2, respectively, resulted in a decrease in *exciplex:LES* ratio (see spectra 2 and 4, respectively).

Effect of structural properties of exci-partners on exciprobe performance

(i) Hydrophobicity and electron-donating properties of the exci-partners. Our experimental data show that based on the relatively crude measure of I_E/I_M, the ability to form an excited state complex decreases in the order pyrene > *N,N*-dialkylamino-naphthalene > *N,N*-dimethylaminobenzyl, if the second exciplex partner is represented by a fixed aromatic group (*e.g.* pyrenyl). Fig. 6A contrasts the emission spectra of SP-1, SP-4 and SP-19

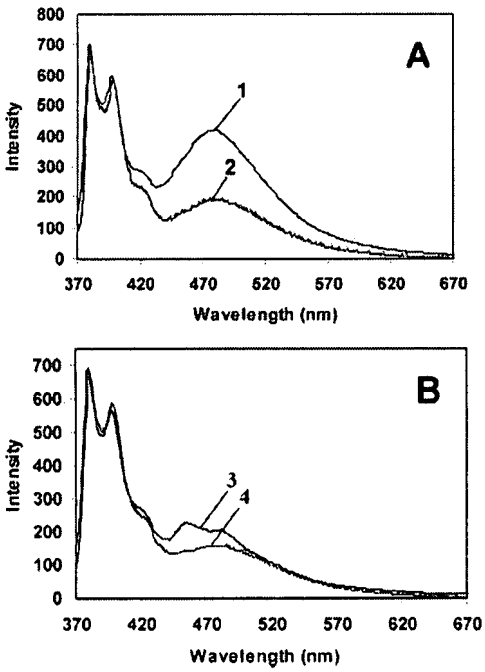


Fig. 5 Comparison of the fluorescence emission of exciplex-forming systems SP-19 *vs.* SP-18 (A, spectra **1** and **2**, respectively) and SP-4 *vs.* SP-2 (**B**, spectra **3** and **4**, respectively) with donor (*N*,*N*-dialkylaminonaphthalene or *N*,*N*-dimethylaminobenzyl) and acceptor (pyrene) partners swapped (see Table 1 for the structures). Spectra were recorded at 10 °C in 80% TFE–10mM Tris buffer, pH 8.4, 0.1 M NaCl with component concentrations of 2.5 μM (1 : 1 : 1); λ_{ext} 350 nm. Due to the very different fluorescence intensity of Pyr-5′-ON1 and ON2-3′-Pyr oligo probes, spectra were scaled against the pyrene LES band at 380 nm to compare *exciplex*:*LES* ratios for the systems studied.

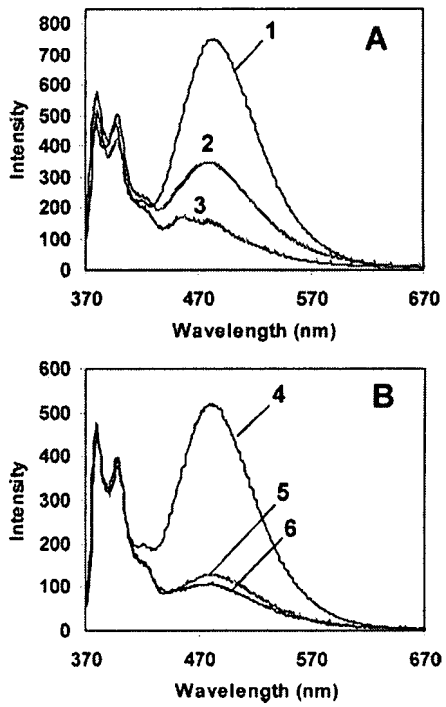


Fig. 6 **A**: Comparison of the emission spectra of systems SP-1, SP-19 and SP-4 with a fixed exci-partner (pyrene) at the 5′-terminal phosphate site of the ON1 oligo probe and different exci-partners (pyrenyl, *N*,*N*′-dialkylaminonaphthaleno or dimethylaminobenzyl, respectively) at the 3′-terminal phosphate site of the ON2 oligo probe. **1**: SP-1 excimer system containing two pyrenyl exci-partners; **2**: SP-19 exciplex system containing *N*,*N*′-dimethylalkylaminonaphthaleno 3′-donor partner; **3** SP-4 exciplex system containing a dimethylaminobenzyl 3′-donor partner. **B**: Comparison of the emission spectra of systems SP-1, SP-18 and SP-2 with a fixed exci-partner (pyrene) at the 3′-terminal phosphate site of the ON2 oligo probe and different exci-partners (pyrenyl, *N*,*N*′-dialkylaminonaphthaleno or dimethylaminobenzyl, respectively) at the 5′-terminal phosphate site of the ON1 oligo probe. **4**: SP-1 excimer system containing two pyrenyl exci-partners; **5**: SP-18 exciplex system containing an *N*,*N*′-dialkylaminonaphthaleno 5′-donor partner; **6**: SP-2 exciplex system containing a dimethylaminobenzyl 5′-donor partner. Spectra were recorded at 10 °C in 80% TFE–10 mM Tris, pH 8.3, 0.1 M NaCl; λ_{ext} 350 nm.

split-probe systems possessing the same exci-partner (pyrene) at the 5′-terminal phosphate of the ON1 oligo probe and different exci-partners (pyrenyl, *N*,*N*′-dialkylaminonaphthaleno or dimethylaminobenzyl, respectively) at the 3′-terminal phosphate of the ON2 oligo probe (spectra are scaled against LES emission band of the pyrene at 380 nm as a reference to allow comparison of *exciplex*:*LES* or *excimer*:*LES* ratios (I_E/I_M) for these systems). It is seen that I_E/I_M is highest for SP-1, possessing pyrene as a second partner. The replacement of 3′-pyrene by 3′-*N*,*N*-dialkylaminonaphthalene (SP-19) resulted in a marked decrease in I_E/I_M. Further replacement of the 3′-partner group by 3′-*N*,*N*-dimethylaminobenzyl (SP-4) resulted in an additional decrease in the relative intensity of the exciplex band. A similar effect was observed when SP-2 and SP-18 (with the reversed location of donor and acceptor groups) were compared with the SP-1 system (Fig. 6B). Detailed evaluation of these types of structural effects requires time-resolved measurements, as indicated by a referee, and such studies are in progress.

(ii) Effect of *N*-alkylation of donor exci-partners. The electron-donating properties of an exci-partner strongly depend on the *nature* and *number* of *N*-alkyl substitution group(s) in the aminobenzyl or aminonaphthaleno aromatic groups. Comparison of SP-18 (with an *N*,*N*-dialkylaminonaphthalene group) and SP-20 (with an *N*-alkylaminonaphthalene donor partner) showed that the relative integral intensity of the exciplex band is slightly higher for SP-18 than for SP-20, and the λ_{em} value for the *N*-methyl donor (485 nm) is at longer wavelength than that for the N–H variant (480 nm) of the naphthalene donor (data not shown).

(iii) Effect of linker structure. One way to improve the performance of DNA-mounted exciprobes is to design linker groups connecting donor and acceptor partners to the oligo probes that could prevent unfavourable interactions of hydrophobic exci-partners with DNA nucleotide bases and/or encourage interactions between exci-partners. Success in this strategy was demonstrated by SP-26, which is a structural analogue of the SP-19 system, but possesses an alternative linker group. In the SP-26 system the ethylenediamine linker connecting 5′-pyrene to the DNA was replaced by a negatively charged aspartate fragment to induce repulsion of the pyrene group from the DNA sugar–phosphate surface. Fig. 7 shows that the *exciplex*:*LES ratio* is significantly enhanced for the SP-26 system (spectrum 2) compared with the original SP-19 system (spectrum 1). However,

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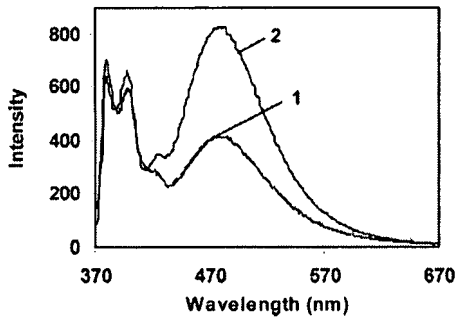


Fig. 7 Comparison of emission spectra of systems SP-19 and SP-26 possessing different linker groups. **1**: SP-19 system with an ethylenediamine linker connecting both exci-partners; **2**: SP-26 system with a negatively charged aspartate linker connecting the 5′-pyrenyl to ON1 oligo probe and ethylenediamine linker connecting the 3′-*N*,*N*′-dialkylaminonaphthaleno exci-partner to ON2 oligo probe. Spectra were recorded at 10 °C (without heating–re-cooling) cycle in 80% TFE–10 mM Tris, pH 8.3, 0.1 M NaCl; λ_{ext} 350 nm.

in pure aqueous solutions SP-26 did not produce an emissive exciplex.

Excimer system SP-25 (a structural analogue of SP-1) with two pyrenyl exci-partners attached to oligo probes *via* aspartate linkers showed good excimer emission in the presence of 80% TFE (see Table 2). However, in aqueous solutions SP-25 produced a fairly weak excimer band (data not shown), which was similar to that observed for SP-1 (Fig. 2) under identical conditions. For excimer systems SP-40 and SP-41 with 'mixed' linker groups (see Table 1 for structures), the *exciplex*:*LES* ratio (I_E/I_M) was rather low even in the presence of TFE (Table 2). A possible explanation could be that pyrenyl partner attached *via* a non-charged ethylenediamine linker may still be involved in competitive interactions with DNA, which discourage its interaction with the second partner and thus reduce excimer emission.

In an attempt to further improve excimer/exciplex emission, we designed system SP-27, SP-28 and SP-29 (see Table 1), which are structural analogues of systems SP-19, SP-18 and SP-1, respectively. Systems SP-27, SP-28 and SP-29 possess an oligopeptide linker (-Ala(Gly)$_4$-) connecting both pyrenyl and *N*,*N*′-dialkylaminonaphthaleno exci-partners to the 5′- or 3′-terminal phosphate groups of the oligo probes. These have the potential for hydrogen bonding between the main chain carbonyl oxygen and amide groups of the peptide linkers, as in β-sheet structures in proteins.[29,30] This could bring the exci-partners close together, favouring efficient excimer/exciplex emission. The exci-partners were thus separated from the nucleotides by five amino acid residues. However, it was shown that SP-19, SP-18 and SP-1 systems, with the short ethylenediamine linker groups, produced much stronger exciplex emission than the corresponding peptide-based versions SP-27, SP-28 and SP-29, respectively. Fig. 8 gives examples of inferior emission of peptide systems SP-27 (curve 1) and SP-29 (curve 3) compared to their structural analogues SP-19 (curve 2) and SP-1 (curve 4), respectively (see the Discussion section). In small organic intramolecular systems, the efficiency of exciplex formation from exciplex partners linked by variable-length CH_2 chains depends on the chain length.[31,32] In such systems, rotational/conformational effects govern the relative location of the exciplex partners. However, in these new DNA constructs the situation is rather different. The present exciplex constructs on DNA are not directly comparable, as the exciplex partners are borne on the tips of two chains (in the context of this

Table 2 Fluorescence emission data collected at 10 °C and melting temperature values obtained for exciplex and excimer systems in 80% TFE/10 mM Tris, pH 8.3, 0.1 M NaCl; λ_{ext} 350 nm except where indicated[a]

System	λ_{em}/nm	I_E/I_M	T_m measured at λ_{em}^{max}
SP-1	486	1.18	27.0 ± 0.5
SP-25	487	0.83	26.0 ± 0.5
SP-2	484	0.22	25.0 ± 1.0
SP-4	480	0.28	26.0 ± 1.0
SP-3	480	0.19	N/D
SP-18	485	0.27	26.0 ± 1.0
SP-19	482	0.61	26.0 ± 1.0[b]
SP-20	480	0.21	N/D
SP-26	484	1.18	N/D
SP-40	481	0.57	25.0 ± 0.5
SP-41	481	0.23	25.0 ± 0.5[c]
SP-27	484	0.45	N/D
SP-28	483	0.48	N/D
SP-29	484	0.23	N/D

[a] N/D: not determined. [b] T_m based on A_{260} was 25.0 °C (ref. 12). [c] The melting temperature measured at λ_{em} 376 nm for the LES of pyrene was 25.0 ± 0.5 °C.

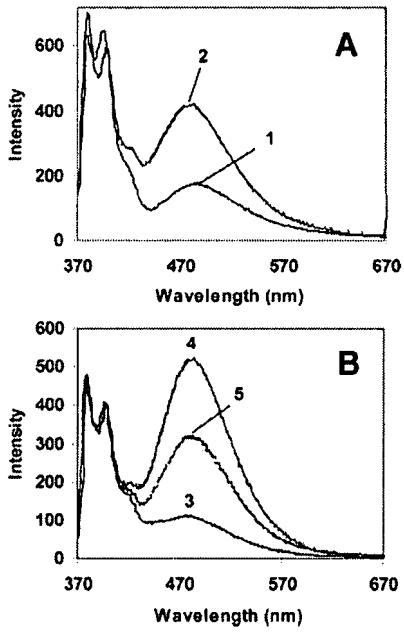


Fig. 8 Effect of an oligopeptide linker (-Ala(Gly)$_4$-) on the performance of exciprobes. **A**: Emission spectrum of the exciplex-based system SP-27 (**1**) with oligopeptide linker (-Ala(Gly)$_4$-) connecting both pyrenyl and *N*,*N*′-dialkylaminonaphthaleno exci-partners to the 5′- and 3′-terminal phosphates of the oligo probes, respectively, *versus* its structural analogue SP-19 (**2**), containing short ethylenediamine linker groups. **B**: Emission spectrum of excimer-based system SP-29 (**3**) with oligopeptide linker (-Ala(Gly)$_4$-) connecting two pyrenyl exci-partners to the 5′- and 3′-terminal phosphate groups of the oligo probes *versus* the structural analogue SP-1 (**4**), containing short ethylenediamine linker groups. Spectrum (**5**) is the emission spectrum of the re-annealed SP-29 system following a heating–re-cooling cycle. Spectra were recorded at 10 °C in 80% TFE–10 mM Tris, pH 8.3, 0.1 M NaCl; λ_{ext} 350 nm.

particular paragraph it is peptides, but not for all the structures we describe) that are connected though a duplex of DNA chains held by a consortium of hydrogen bonds and stacking interactions.

For excimer system SP-29, containing two pyrenyl exci-partners attached to oligo probes *via* the peptide linker, it was possible to improve exciplex emission by a heating–re-cooling cycle (curve 5).

Melting profiles

Melting temperature profiles were obtained for the studied split-probe systems (Table 1) by monitoring fluorescence emission intensity as function of temperature increase (from 5 °C to 60 °C) following detection at both exciplex λ_{em} (480 nm) and LES λ_{em} (376 nm). Fig. 9 shows melting temperature profiles for the SP-41 system as a typical example of such thermal denaturation. The sigmoidal temperature dependence of the exciplex emission signal disappearance (monitored at 480 nm; curve 1) and of the LES emission band re-appearance (monitored at 376 nm; curve 2) corresponds to a DNA duplex melting profile typical of the dsDNA to ssDNA transition. These data provide further evidence of tandem duplex formation, showing only one transition, consistent with literature findings that duplex structures containing a backbone nick in one of the strands melt cooperatively.[33–35] T_m values, along with summarised data for fluorescence emission maxima and I_E/I_M values, are collected in Table 2.

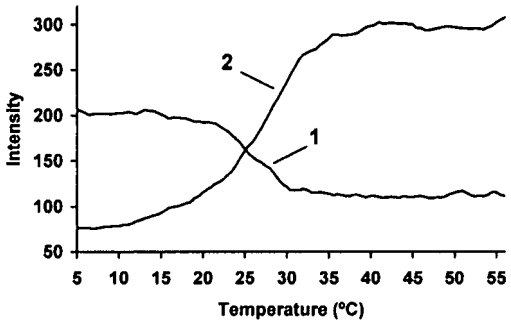


Fig. 9 Melting curves for the excimer system SP-41 (ON1-5′-AspPyr + ON2-3′-Pyr + Target), plotting relative fluorescence emission at 480 nm (1, λ_{ex} 350 nm) and at 376 nm (2, λ_{ex}, 340 nm) against temperature in 80% TFE/Tris buffer (10 mM Tris, 0.1 M NaCl, pH 8.3); component concentration 2.5 μM.

Discussion

Split-probe approach for exciplex-based detection of nucleic acids

In the last year two approaches using DNA-mounted exciplexes have been reported for detection of nucleic acids. In addition to our preliminary communication of the approach in the present study there is a report by Kashida *et al.* who demonstrated efficient exciplex emission in pure aqueous solution for the system with exciplex partners introduced into the middle of DNA.[14] In one specific example, exciplex partners (pyrene and *N,N*-dimethylaniline moieties) were located *face-to-face* in the DNA duplex. However, this approach requires both the oligo-probe and the target sequence to be labelled by exciplex chromophores, which may rather complicate bioassay conditions. Strong exciplex emission was also observed when both exciplex partners were consecutively positioned in the middle of the single DNA oligo probe. However, in the case of successive location of exciplex partners, even a single-stranded oligo-probe produced exciplex emission, giving an undesirable background signal. For detection of single-base insertions and deletions, the exciplex partners were separated by one nucleotide residue to block interaction between them and thus prevent exciplex emission on hybridization with perfect match. Hybridisation with the target containing a single-base deletion resulted in looping of the unhybridised probe region containing exciplex partners, which induced interaction between the two exciplex chromophores accompanied by exciplex emission. Although this research clearly demonstrates that exciplex emission can be observed even in aqueous solution, the disadvantage of this method was a prerequisite of secondary structure formation on hybridization (*e.g.* looping of the fragment possessing exciplex partners). Also, although this method succeeded in detection of insertions and deletions, the application of this approach to the DNA mismatch detection has not yet been reported for it.

The alternative split-probe approach of the present study uses externally oriented DNA-mounted exciplexes, from two separate exciprobes designed to produce exciplex emission by being specifically assembled *in situ* by hybridisation with a bio-target. In this case, a unique fluorescence signal can be generated *only* after specific self-assembly of signal-silent exciplex components with a perfectly matched target. The advantage of the exciplex-based split-probe approach is low or zero background, ultra-biospecificity, and ability to detect DNA mismatches.

Melting temperature studies

T_m profiles for split-probe systems (Table 1) were obtained by monitoring fluorescence intensity from 5 to 60 °C for both exciplex/excimer λ_{em} (480 nm) and LES λ_{em} (376 nm). Melting curves presented in Fig. 9 for the excimer system SP-41 provide a pictorial example of thermal denaturation of such split-probe systems in the presence of 80% TFE. Melting profiles reflect thermal denaturation of the duplex, causing the dissociation of the 8-mer split-probes from the duplex, which results in separation of exci-partners. This is accompanied by eventual disappearance of exciplex/excimer signal and subsequent re-appearance of the LES fluorescence band, previously quenched within the excited state complex. The sigmoidal nature of the melting curves, typical of the transition of dsDNA to ssDNA, provides evidence that presence of co-solvent (80% TFE) does not disrupt duplex formation. However, T_m values in the presence of 80% TFE were typically 10–11 °C lower than in purely aqueous solutions, demonstrating the destabilisation effect of the TFE co-solvent, presumably attributable to a decrease of hydrophobic stacking interactions of nucleotide bases, or a change in duplex structure. T_m values calculated from the melting profiles (Table 2) were very similar for all split-probe systems studied, ranging between 25.0 ± 0.5 and 27.0 ± 0.5 °C, reflecting similarity in the duplex stabilities for these systems. SP-1 was the most stable (T_m = 27.0 ± 0.5 °C), presumably due to the presence of two pyrenyl residues, which are known to stabilise DNA duplexes.[33,34]

TFE co-solvent for exciplex/excimer signal development

In model organic systems corresponding to the SP-19 and SP-4 cases, solvent polarity was found to have very little effect on the

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emission wavelength (λ_{em}) of the LES of the pyrenyl partner. In contrast, exciplex emission showed a much greater dependence on solvent dielectric constant, with λ_{em} varying over a range of nearly 100 nm on going from cyclohexane (ε 2.0) to *N*-methylformamide (ε 182).[11] This is consistent with the extensive literature showing that solvent polarity is crucial to the properties of exciplexes.[10] However, exciplex emission is more than just a matter of solvent polarity in these DNA-mounted exciplex cases because out of the many solvents tested only a few were able to induce exciplex emission and of these, TFE was especially favourable. Exciplex emission was not observed for various co-solvents (50 and 70% v/v tested), *viz.* methanol, ethanol, acetone, hexafluoropropan-2-ol or tetrafluoropropan-1-ol. Exciplex emission for the fully assembled system was strongly and specifically enhanced by trifluoroethanol (TFE), and weakly by ethylene glycol and ethylene glycol dimethyl ether (~5 and 10-fold less than TFE, respectively).[13] The origins of the rather specific effects of TFE on exciplex emission for DNA systems is difficult at this stage to explain unambiguously and probably arises from a combination of factors. TFE has a particular ability to induce a structural transition in DNA favouring a shift from B-like to A-like.[36] However, by changing the hydrophobicity of the environment, this co-solvent also regulates the excited-state attraction of the exci-partners through (i) affecting their relative attraction for each other in the excited state (and perhaps also in the ground state) and (ii) through the long-established stronger exciplex emission in lowered polarity environments.[9,37-39]

Hydrophobic interactions between donor and acceptor in model intramolecular organic exciplexes with the same types of donor and acceptor pairs as in the present study stabilise a pre-formed stacking structure detected by cross-peaks between them in NOESY NMR experiments.[11] In polar media, such as for the DNA systems of the present study, this would enhance the probability of the exci-partners forming their excited-state complex on photo-excitation. Our observations show that hydrophobic properties of the exci-partners significantly enhance exciplex emission (see next section). However, aromatic nucleotide bases may competitively interact with exci-partners *via* formation of non-specific hydrophobic contacts with donor or/and acceptor partners thus preventing exciplex formation. Our NMR and UV-visible data[33,34] provide strong evidence of strong hydrophobic interactions of a pyrenyl group with the DNA part of a split-probe. This competition would act to discourage favourable interactions between exci-partners, especially if one of them is not hydrophobic enough to compete with pyrene–DNA interactions. Therefore, the exci-partners should possess similar hydrophobic properties to increase the chance of exciplex/excimer formation. The use of specific co-solvents has also been noted in other systems that operate by the principles of Fig. 1. Thus, Masuko *et al.* studied an excimer-based split-probe system using DMF as a hybridisation co-solvent, in analogy to the use of formamide to weaken base-pairing, and found that an optimal amount was 20% v/v.[5] The DMF was also proposed to influence fluorescence quantum yields through an effect on pyrene–solvent dipole–dipole interactions.[5]

Hydrophobic and electron donor/acceptor properties of the exci-partners. Our studies of DNA-mounted exciplex/excimer split-probe systems show that a reasonable compromise in hydrophobic properties of exci-partners has to be achieved for successful exciplex/excimer formation. The excimer system SP-1 possessing two pyrenyl exci-partners produced the strongest signal at 480 nm (both in absolute terms and, as shown in Fig. 6, when normalised to the LES of pyrene) in comparison with SP-19, SP-18, SP-4 and SP-2 exciplex systems with the second exci-partner replaced by *N*,*N*-dimethylaminonaphthalenyl or *N*,*N*-dimethylaminophenyl. However, it is hard to directly compare excimers and exciplexes in view of the different degrees of charge transfer involved in their emissive excited states. Formation of excited-state complexes also depends on the ionisation potentials of the partners.

Effect of linker group on exciplex formation. The linkers connecting the exci-partners to the oligo-probes strongly affect the ability of donor and acceptor to interact and thus play an important role in excimer/exciplex formation. Our studies demonstrate that correctly designed linker groups significantly enhance exciplex formation. Replacement of the neutral ethylenediamine linker with a negatively charged aspartate fragment (*e.g.* system SP-26) notably enhanced exciplex emission, presumably due to the induced repulsion of the pyrene group from the negatively charged sugar–phosphate surface of DNA (Fig. 8). Excimer system SP-25 with aspartate linker groups connecting both pyrene partners to the oligo probes showed detectable excimer emission even in pure aqueous solutions, similar to that observed for SP-1 (Fig. 2).

Another attempt in improving exciplex formation was based on use of peptide fragments as linker groups to induce chain interactions between the two linkers, thereby bringing the exci-partners close and stabilising their interactions. This should have resulted in an increase in excimer/exciplex emission relative to the short linker systems. However, exciplex emission of peptide-based systems was lower than the short-linker analogues. It is therefore possible that no defined structure formed between the two peptide linker systems. β-Sheet formation in proteins and peptides occurs intramolecularly through formation of a β-hairpin.[40] However, the peptide linkers in this study are separate molecules, and therefore may not form a β-sheet, especially in TFE media. While the junction of the split oligos could serve the role of the hairpin, it will be necessary to replace the conformationally unrestrained glycine residues, which are known to destroy secondary structure.[29] Although interaction of the exci-partners did occur within peptide-based systems SP-27, SP-28 and SP-29, exciplex emission between exci-partners was reduced, presumably due to the high conformational flexibility of these long linker groups reducing the probability of collisions between exci-partners.

Conclusions

This research has investigated contributions from various factors on exciplex performance within DNA tandem systems. These factors include the relative hydrophobicities of the partners used in the exciprobes, the medium in which the systems are studied, the electron-donating and electron-accepting characteristics of the exci-partners, and the structures and charge distributions of linker groups. Careful design of exci-partners and linker groups, as well as appropriate experimental design (*e.g.* media, use of a heating–re-cooling cycle, *etc.*) significantly enhances exciplex/excimer emission signal for DNA-mounted exciplexes.

Methods

Pyren-1-ylmethylamine hydrochloride, (4-(aminomethyl)phenyl)-dimethylamine hydrochloride, *N*-(naphthalen-1-yl)ethane-1,2-diamine dihydrochloride and (4-(dimethylamino)phenyl)acetic acid for the preparation of ON1-5′-Pyr, ON2-3′-Pyr, ON1-5′-DMA, ON2-3′-DMA, ON1-5′-NHNp, ON2-3′-NHNp and ON1-5′-C7DMA, respectively, were purchased from Sigma-Aldrich Chemical Co., (Poole, Dorset, UK). *N*-Methyl-*N*-(naphthalen-1-yl)ethane-1,2-diamine dihydrochloride for the preparation of ON1-5′-NmeNp and ON2-3′-NmeNp oligo probes was synthesised as described.[13] Oligodeoxynucleotides were purchased from Eurogentec (Liege, Belgium) and Sigma-Aldrich-Proligo (Paris, France). Cetyltrimethylammonium bromide, triphenylphosphine, dipyridyldiphosphate, dimethylaminopyridine, triethylamine, lithium perchlorate, dimethylformamide, hydroxybenzotriazole, *N*-methylmorpholine and dicyclohexylcarbodiimide were purchased from Sigma-Aldrich Chemical Co., (Poole, Dorset, UK). H-BocAla(Gly)$_4$-OH was purchased from Bachem (St Helens, UK).

Attachment of H-BocAla(Gly)$_4$-OH peptide linker to pyren-1-ylmethylamine and *N*-methyl-*N*-(naphthalen-1-yl)ethane-1,2-diamine

H-BocAla(Gly)$_4$-OH was attached to pyren-1-ylmethylamine and *N*-methyl-*N*-(naphthalen-1-yl)ethane-1,2-diamine according to Scheme 1 for subsequent preparation of ON1-5′-Pept-Pyr, ON2-3′-Pept-Pyr, ON1-5′-Pept-NMeNp and ON3-3′-Pept-NMeNp oligo probes.

Pyren-1-ylmethylamine hydrochloride (**VI**) (0.25 g, 0.82 mmol) or *N*-methyl-*N*-(naphthalen-1-yl)ethane-1,2-diamine (**VII**) (0.20 g, 0.82 mmol) and triethylamine (0.11 ml, 0.82 mmol) were dissolved in DMF (15 mL) and stirred at room temperature for 30 min. H-BocAla(Gly)$_4$-OH (0.25 g, 0.82 mmol), hydroxybenzotriazole (0.11 g, 0.82 mmol) and *N*-methylmorpholine (0.091 mL, 0.82 mmol) were then added, the reaction mixture cooled in an ice bath and dicyclohexylcarbodiimide (0.18 g, 0.86 mmol) added. The reaction mixture was stirred in the ice bath for 1 h, then refluxed for several hours, and stirred at room temperature overnight. The mixture was filtered to remove the urea formed, and DMF removed *in vacuo*. The residue was dissolved in ethyl acetate (50 mL) and extracted with 25 mL portions of saturated sodium hydrogen carbonate, 10% citric acid, sodium hydrogen carbonate and finally water. The organic layer was dried over anhydrous sodium sulfate and the solvent removed *in vacuo* to give the crude product **IX** or **X** as an orange solid (see Scheme 1).

To remove the Boc protecting group, a solution of **IX** or **X** in 50% TFA and 2% triethylsilane in methanol (total volume 20 mL) was stirred for several hours at room temperature until TLC (ethyl acetate–methanol 60 : 40) showed complete reaction. Solvents and by-products were removed *in vacuo* and the product **XI** or **XII** recrystallised from hexane and methanol as a slightly orange solid, which was characterised by ^{1}H NMR spectroscopy.

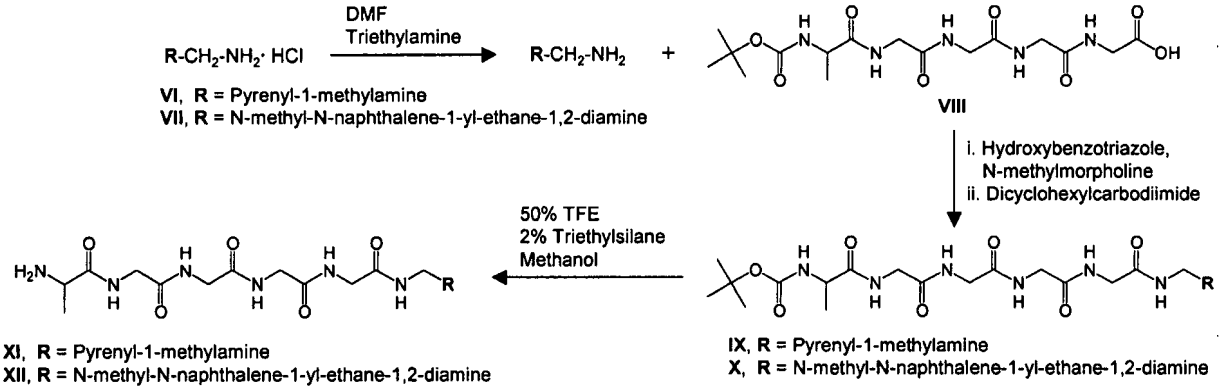
^{1}H NMR of **XI** (MeOD): δ_H 1.46 (d, 3H, CH_3, Ala), 3.87–3.96 (m, 8H, 4 × CH_2, Gly), 4.01–4.12 (m, 1H, CH, Ala), 5.14 (s, 2H, -CH_2NH- of pyrenemethylamino group), 8.00–8.36 (m, 9H, 9 × Ar-H, pyrene).

^{1}H NMR of **XII** (D_2O): δ_H 1.41 (d, 3H, CH_3 of Ala), 2.86 (s, 3H, -NCH_3), 3.43 (m, 2H, -CH_2NHCO-), 3.61 (m, 2H, -CH_2NMe-), 3.87–3.82 (m, 8H, 4 × CH_2, Gly), 4.01–4.12 (m, 1H, CH, Ala), 7.22–8.32 (m, 7H, 7 × ArH, naphthyl).

Conjugation of exci-partners to oligonucleotide probes ON1 and ON2

In general, attachment of exci-partners bearing an aliphatic amine functional group to the 5′- or 3′-terminal phosphate group of oligonucleotide probes ON1 and ON2 followed by purification and characterisation was achieved according to the published protocols.[13] β-(Pyren-1ylmethylaminocarboxamide)aspartic acid (a gift from Dr Ben Law and AnMat Co., Manchester) was attached similarly through its α-amino group, to give ON1-5′-AspPyr and ON2-3′-AspPyr. ON1-5′-Pyr and ON2-3′-NMeNp oligo probes were characterised as described elsewhere.[13]

ON1-5′-NMeNp: ^{1}H NMR (D_2O): δ_H 1.77–1.86 (s, 12H, 4 × CH_3′ 4 × dT), 2.15–2.92 (m, 16H, 8 × H2′ and 8 × H2″ sugar ring protons), 2.79 (s, 3H, NCH_3), 3.22 (t, 2H, -NHCH_2-), 3.43 (t, 2H, -NHCH_2-), 3.60–4.60 (m, 24H, 8 × H4′, 8 × H5′ and 8 × H5″ sugar ring protons), 5.4–6.40 (m, 8H, 8 × H1′ of sugar ring and d, 1H, H5 of dC), 7.16–8.14 (m, 7H, 7 × Ar-H, naphthyl, s, 7H, 7 × Ar-H from each dG (×3) and dT (×4); d, 1H, H6 of dC). The H3′ sugar ring proton region (4.90–5.20 ppm) was not analysed due to suppression of residual water signal at 4.8 ppm. The UV-visible absorption spectra of ON1-5′-NMeNp showed a shoulder at 310 nm on the 260 nm absorption band, indicating the presence of naphthalene.



Scheme 1 Attachment of H-BocAla(Gly)$_4$-OH peptide linker to pyren-1-ylmethylamine and *N*-methyl-*N*-(naphthalen-1-yl)ethane-1,2-diamine.

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ON2-3′-Pyr: ^{1}H NMR (D_2O): δ_H 1.66–1.70 (s, 9H, 3 × CH_3 of 3 × dT), 2.13–2.93 (m, 16H, 8 × H2′ and 8 × H2″ sugar ring protons), 3.60–4.60 (m, 24H, 8 × H4′, 8 × H5′, 8 × H5″ sugar ring protons; bs, 2H, -NHCH_2-), 5.4–6.40 (m, 8H, 8 × H1′ of sugar; d, 2H, 2 × H5 of 2 × dC), 6.91–8.34 (m, 9H, 9 × Ar-H of pyrene; s, 7H, 7 × Ar-H from nucleotide bases (1 from each dG (×2) and dT (×3), 2 from dA); d, 2H, H6 of dC (×2)). The resonance area of H3′ sugar ring protons (4.90–5.20 ppm) was not analysed due to suppression of residual water signal at 4.8 ppm. The presence of pyrene alters the UV-visible absorption spectrum of ON2-3′-Pyr, leading to an additional band around 345 nm, and altering the band at 260 nm. The ratio between the absorbances at 260 and 345 nm was around 3.0, typical of mono-pyrene-substituted 8-mer oligonucleotides.

ON1-5′-DMA: ^{1}H NMR (D_2O): δ_H 1.76–1.87 (s, 12H, 4 × CH_3 of 4 × dT), 2.15–2.92 (m, 16H, 8 × H2′ and 8 × H2″ sugar ring protons), 2.60–2.63 (d, 6H, -N(CH_3)$_2$), 3.60–4.60 (m, 24H, 8 × H4′, 8 × H5′, 8 × H5″ sugar ring protons; bs, 2H, -NHCH_2-), 5.4–6.40 (m, 8H, 8 × H1′ of sugar ring; d, 1H, H5 of dC), 7.16–8.14 (s, 7 × Ar-H from each dG (×3) and dT (×4); d, 1H, H6 of dC) 6.62–7.02 (d, 4H, 4 × Ar-H of *N*,*N*-dimethylaminobenzyl). The H3′ sugar ring proton region (4.90–5.20 ppm) was not analysed due to suppression of residual water signal at 4.8 ppm.

ON2-3′-DMA: ^{1}H NMR (D_2O): δ_H 1.65–1.71 (s, 9H, 3 × CH_3 of 3 × dT), 2.15–2.92 (m, 16H, 8 × H2′ and 8 × H2″ sugar ring protons), 2.60–2.63 (d, 6H, -N(CH_3)$_2$); 3.60–4.60 (m, 24H: 8 × H4′, 8 × H5′, 8 × H2″ sugar ring protons; bs, 2H, -NHCH_2); 5.4–6.40 (m, 8H, 8 × H1′ of sugar ring; d, 2H, 2 × H5 of 2 × dC), 6.62–7.02 (d, 4H, 4 Ar-H of *N*,*N*-dimethylaminobenzyl), 6.91–8.34 (s, 7H, Ar-H from nucleotide bases (1 from each dG (×2), and dT (×3), 2 from dA); d, 2H, H6 of dC (×2)). The resonance region of H3′sugar ring protons (4.90–5.20 ppm) was not analysed due to suppression of residual water signal at 4.8 ppm.

ON1-5′-Pept-Pyr: ^{1}H NMR (D_2O): δ_H 1.00–1.03 (d, 3H, CH_3 of alanine), 1.50–1.64 (s, 12H, 4 × CH_3 of 4 × dTs), 2.15–2.92 (m, 16H, 8 × H2′ and 8 × H2″ sugar ring protons), 3.87–3.96 (m, 8H, 4 × CH_2 of 4 Gly residues), 3.60–4.60 (m, 32H, 8 × H4′, 8 × H5′, 8 × H5″ sugar ring protons, 4 × CH_2 of 4 Gly residues; bs, 2H, -NHCH_2-), 5.4–6.40 (m, 8H, 8 × H1′ of sugar ring; d, 1H, H5 of dC), 6.60–8.16 (m, 9H, 9 × Ar-H from pyrene; s, 7H, 7 × Ar-H from each dG (×3) and dT (×4); d, 1H, H6 of dC). The H3′ sugar ring proton region (4.90–5.20 ppm) was not analysed due to suppression of residual water signal at 4.8 ppm. The presence of pyrene alters the UV-visible absorption spectrum of ON1-5′-Pept-Pyr, leading to an additional band around 345 nm, and altering the band at 260 nm. The ratio between the absorbances at 260 and 345 nm was around 3.0, typical of mono-pyrene-substituted 8-mer oligonucleotides.

ON2-3′-Pept-Pyr: ^{1}H NMR (D_2O): δ_H 1.35–1.38 (d, 3H, CH_3 of Ala), 1.65–1.66 (s, 9H, 3 × CH_3 of 3 × dT), 2.15–2.92 (m, 16H, 8 × H2′ and 8 × H2″ sugar ring protons), 3.60–4.60 (m, 32H, 8 × H4′, 8 × H5′, 8 × H5″ sugar ring protons, 4 × CH_2 of 4 Gly residues; d, 2H, -NCH_2); 5.4–6.40 (m, 8H, 8 × H1′ of sugar ring; d, 2H, 2 × H5 of 2 × dC), 6.91–8.34 (m, 9H, 9 × Ar-H from pyrene; s, 7H, 7 × Ar-H from nucleotide bases (1 from each dG (×2) and dT (×3), 2 from dA); d, 2H, H6 of dC (×2)). The H3′ sugar ring proton region (4.90–5.20 ppm) was not analysed due to suppression of the residual water signal at 4.8 ppm. The presence of pyrene alters the UV-visible absorption spectrum of ON2-3′-Pept-Pyr, leading to an additional band around 345 nm, and altering the band at 260 nm. The ratio between the absorbances at 260 and 345 nm was around 3.0, typical of mono-pyrene-substituted 8-mer oligonucleotides.

ON1-5′-Pept-NMeNp: ^{1}H NMR (D_2O): δ_H 1.00–1.03 (d, 3H, CH_3 of Ala);1.54–1.64 (s, 12H, 4 × CH_3 of 4 × dT), 2.15–2.92 (m, 16H, 8 × H2′ and 8 × H2″ sugar ring protons), 2.60 (s, 3H, N-CH_3), 3.22 (t, 2H, -NHCH_2-), 3.43 (t, 2H, -NHCH_2-), 3.60–4.60 (m, 32H, 8 × H4′, 8 × H5′, 8 × H5″ sugar ring protons and 4 × CH_2 of 4 Gly residues), 5.4–6.40 (m, 8H, 8 × H1′ of sugar ring; d, 1H, H5 of dC), 6.92–8.14 (m, 7H, 7 × Ar–H, naphthyl; s, 7H, 7 × Ar-H from each dG (×3) and dT (×4); d, 1H, H6 of dC). The H3′ sugar ring proton region (4.90–5.20 ppm) was not analysed due to suppression of residual water signal at 4.8 ppm. The UV-visible absorption spectra of ON1-5′-Pept-NMeNp showed a shoulder at 310 nm on the 260 nm absorption band, indicating the presence of naphthalene.

ON2-3′-Pept-NMeNp: 1H NMR (δH, D_2O): δ_H 1.35–1.38 (d, 3H, CH_3 of Ala), 1.42–1.64 (s, 9H, 3 × CH_3 of 3 × dT), 2.15–2.92 (m, 16H, 8 × H2′ and 8 × H2″ sugar ring protons), 2.67 (s, 3H, N-CH_3), 3.22 (t, 2H, -NHCH_2-), 3.43 (t, 2H, -NHCH_2-), 3.60–4.60 (m, 32H, 8 × H4′, 8 × H5′, 8 × H5″ sugar ring protons; 4 × CH_2 of 4 Gly residues); 5.4–6.40 (m, 8H, 8 × H1′ of sugar; d, 2H, 2 × H5 of 2 × dC), 6.91–8.34 (m, 7H, 7 × Ar-H of *N*′-methyl-*N*′-naphthyl; s, 7H, 7 × Ar-H from nucleotide bases (1 from each dG (×2) and dT (×3), 2 from dA); d, 2H, H6 × 2 of dC (×2)). The resonance area of H3′ sugar ring protons (4.90–5.20 ppm) was not analysed due to suppression of residual water signal at 4.8 ppm. The UV-visible absorption spectra of ON2-3′-Pept-NmeNp showed a shoulder at 310 nm on the 260 nm absorption band, indicating the presence of naphthalene.

ON1-5′-NHNp: ^{1}H NMR (D_2O): δ_H 1.77–1.86 (s, 12H, 4 × CH_3′ 4 × dT), 2.15–2.92 (m, 16H, 8 × H2′ and 8 × H2″ sugar ring protons), 3.22 (t, 2H, -NHCH_2-), 3.43 (t, 2H, -NHCH_2-), 3.60–4.60 (m, 24H, 8 × H4′, 8 × H5′ and 8 × H5″ sugar ring protons), 5.4–6.40 (m, 8H, 8 × H1′ of sugar ring; d, 1H, H5 of dC), 7.16–8.14 (m, 7H, 7 × Ar-H, naphthyl; s, 7H, 7 × Ar-H from each dG (×3) and dT (×4); d, 1H, H6 of dC). The H3′ sugar ring proton region (4.90–5.20 ppm) was not analysed due to suppression of residual water signal at 4.8 ppm. The UV-visible absorption spectra of ON1-5′-NHNp showed a shoulder at 310 nm on the 260 nm absorption band, indicating the presence of naphthalene.

Preparation of ON1-5′-C7DMA oligonucleotide probe

Synthesis of the ON1-5′-C7DMA oligonucleotide probe containing the heptane-1,7-diamine linker (C-7) was achieved using two-step conjugation, *viz.*, initial coupling of heptane-1,7-diamine to the oligonucleotide followed by attachment of (4-(dimethylamino)phenyl)acetic acid to the free aliphatic amino group of the linker. Oligonucleotide ON1 (0.5 μmol, 35 ou_{260}) was dissolved in H_2O (100 μL) and precipitated by addition of 4% aqueous cetyltrimethylammonium bromide (20 μL). The sample was then centrifuged (5 min, 10 000 rpm) and the procedure repeated until there was no apparent precipitation after the addition of the cetyltrimethylammonium bromide. The supernatant was carefully removed and the pellet washed by water and dried under vacuum overnight.

A solution of oligonucleotide as the cetyltrimethylammonium salt in DMF (~150 μL) was treated with 2,2′-dipyridyl disulfide

(6.6 mg, 30 μmol) and triphenylphosphine (8 mg, 30 μmol) for 10 min, followed by treatment by 4-dimethylaminopyridine (6 mg, 50 μmol) for 10 min at 25 °C. The activated oligonucleotide was then precipitated using 2% $LiClO_4$ in acetone (2 mL), and the dried pellet dissolved in 1 M aqueous heptane-1,7-diamine (150 μL) and left for 1 h. On completion of reaction the oligonucleotide material was precipitated using 2% $LiClO_4$ in acetone (2 mL), dissolved in H_2O (100 μL) and purified by reverse-phase HPLC. The cetyltrimethylammonium salt of the oligonucleotide with the attached heptanediamine linker was prepared as described above and dissolved in anhydrous DMF (100 μL).

To a solution of (4-(dimethylamino)phenyl)acetic acid (17.9 mg, 100 μmol) in anhydrous DMF (100 μL), BOP (88.5 mg, 200 μmol) and 4-(dimethylamino)pyridine (24.4 mg, 200 μmol) were added and the mixture left for 30 min at 50 °C. The solution of oligonucleotide with attached heptanediamine linker was added and the resulting mixture left to react at 50 °C for 1 h. The reaction mixture was precipitated by addition of 2% $LiClO_4$ in acetone (2 mL), the supernatant discarded, the pellet dried, dissolved in H_2O (100 μL) and purified by reverse-phase HPLC.

ON1-5′-C7DMA: ^{1}H NMR (D_2O): δ_H 1.29–1.56 (m, 10H, 5 × $-CH_2-$) 1.76–1.87 (s, 12H, 4 × CH_3 of 4 × dT), 2.15–2.92 (m, 18H, 8 × H2′ and 8 × H2″ sugar ring protons; 2H, $-CH_2NH-$), 2.60–2.63 (d, 6H, $-N(CH_3)_2$), 3.21 (t, 2H, $-CH_2NHCO-$), 3.44 (s, 2H, $-CH_2CO-$) 3.60–4.60 (m, 24H, 8 × H4′, 8 × H5′, 8 × H5″ sugar ring protons); 5.4–6.40 (m, 8H, 8 × H1′ of sugar ring; d, 1H, H5 of dC), 6.62–7.02 (d, 4H, 4 Ar-H of *N*,*N*-dimethylaminobenzyl), 7.16–8.14 (s, 7 × Ar-H from each dG (×3) and dT (×4); d,1H, H6 of dC). The H3′ sugar ring proton region (4.90–5.20 ppm) was not analysed due to suppression of residual water signal at 4.8 ppm.

HPLC

Reverse-phase HPLC purification of oligonucleotides was performed using an HPLC Holochrome 302 (Gilson) chromatograph equipped with a C18 column (Vydac™, particle size 10 μm, inner diameter 10 mm, length 250 mm, pore size 300 Å) with elution using an increasing gradient (0–50%) of acetonitrile in water (fraction detection at 260 nm). In some cases HPLC purifications of probes were performed on an Agilent 1100 Series HPLC system, consisting of a quaternary pump with solvent degasser, a diode-array module for multi-wavelength signal detection using an Agilent 1100 Series UV-visible detector and an Agilent 1100 Series fluorescence detector for on-line acquisition of excitation/emission spectra. The system had a manual injector and thermostatted column compartment with two heat exchangers for solvent pre-heating. The HPLC system was operated through Agilent HPLC 2D ChemStation Software. Depending on the purification performed, the columns used were: Zorbax Eclipse X DB-C8 column (length 25 cm, inner diameter 4.6 mm, particle size 5 μm), or a Luna C18 (2) column (length 25 cm, inner diameter 4.6 mm, particle size 5 μm) with elution using an increasing gradient (0–50%) of acetonitrile in water (detection at 260, 280, and 340 nm).

Fluorescence spectroscopy

UV-Visible absorption spectra were measured at 20 °C on a Cary-Varian 1E UV-Visible spectrophotometer with a Peltier-thermostatted cuvette holder and Cary 1E operating system/2 (version 3) and the Cary 1 software. Quantification of the oligonucleotide components used millimolar extinction coefficients (ε_{260}) of 70.2 for ON1, 79.7 for ON2 and 169.4 mM^{-1} cm^{-1} for the 16-mer target. For the oligonucleotide probes ON1-5′-Pyr and ON2-3′-Pyr millimolar extinction coefficients (ε_{260}) were corrected taking into account millimolar extinction coefficients of pyren-1-ylmethylamine at 260 nm (ε_{260} for pyren-1-ylmethylamine in water was determined to be 15.34 mM^{-1} cm^{-1}). Fluorescence excitation and emission spectra were recorded on a thermostatted Shimadzu RF-5301 spectrofluorophotometer, or a Varian Eclipse Fluorescence Spectrophotometer with a Peltier-thermostatted cuvette holder. For comparative purposes the standard component concentration of tested samples in the cuvette was 2.5 μM. Spectra were recorded in Tris buffer (10 mM Tris, 0.1 M NaCl, at pH 8.5) containing various percentages of TFE or other co-solvents (0–80%) using a 2 mL or 100 μL thermostatted 4-sided quartz cuvette. Excitation wavelengths for both monomer and exciplex/excimer emission were optimised in each experiment and ranged from 340 to 350 nm. In most cases slitwidths were set from 5 to 10 nm both for excitation and emission spectra, depending on the intensity of emission. "Automatic shutter-on" regime was used to minimise photodegradation of compounds in the cuvette. All spectra were corrected for buffer, TFE and/or naphthalene emission as appropriate at the specific temperature and wavelength. Fluorescence units shown on figures are arbitrary fluorescence intensity units, except where spectra were scaled for comparison, in which cases appropriate ratios of them were used. In some cases spectra were scaled to the pyrene LES emission at 380 nm of the pyrene-bearing probe to facilitate visualisation of changes in the ratio between the pyrene LES and the excimer/exciplex band. The ratio between the emission intensity of the excimer/exciplex band at 480 nm and the LES band at 380 nm (I_E/I_M) was also calculated for each spectrum.

Duplex formation

1 mL volume. The complex was formed by sequential addition of aliquots (2.5 μL) of the components from stock solutions (1 mM) to Tris buffer (1 mL of 10 mM Tris, pH 8.30, 100 mM NaCl) containing the appropriate amount of TFE (0–80%) at 10 °C. Components (molar ratio 1 : 1 : 1) were added in the order: ON1-5′-X, ON2-3′-Y, then target. The concentration of each component in the cuvette was 2.5 μM. On formation of the full complex, the system was allowed to equilibrate for 10 min at 10 °C or 5 °C, and emission spectra then recorded at 3 min intervals. For the heating–re-cooling cycle, the cuvette containing all components was heated to to 70 °C for 5 min and allowed to cool back to 10 °C over 40 min. Emission spectra were then recorded at 3 min intervals until exciplex emission did not increase further (this usually occurred after 9 min). All spectra were baseline- and TFE-corrected. As the emission from naphthalene is a broad band (λ_{max} 436 nm), spectra were recorded separately for systems containing a naphthalene-bearing probe: the potential naphthalene contribution was thus shown to be very small under the conditions of excitation used.

100 μL volume. Studies were carried out to show that the types of measurement described above for 1 mL reaction volumes could also be used for smaller samples. The emission spectrum

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of the full SP-19 system (ON1-5'Pyr + ON2-3'NmeNp + 16-mer oligonucleotide target) was recorded for a 1 mL sample in the cuvette (excitation 350 nm, 5 nm slitwidth, medium PMT detector voltage). An aliquot (50 μL) of this solution was pipetted into a microcuvette and the emission spectrum recorded under the same conditions, but with a high PMT detector voltage. The experiment was repeated several times, and similar spectral results were always obtained with both sample volumes (data not shown). Duplex formation was also studied on small volumes as follows. The complex was formed by sequential addition of aliquots (1.25 μL) of the components from stock solutions (0.2 mM) to Tris buffer (100 μL of 10 mM Tris, pH 8.40, 100 mM NaCl) containing the appropriate amount of TFE (0–80% v/v) at 5 °C. Components (molar ratio 1 : 1 : 1) were added in the order: ON1-5'Pyr, ON2-3'NMeNp, then target. The final concentration of each component in the cuvette was 2.5 μM. On formation of the full complex, the system was allowed to equilibrate for 5–10 min at 5 °C, and emission spectra recorded at 3 min intervals. The cuvette was then heated to 60 °C for 5 min and allowed to cool back to 5 °C over 40 min. Emission spectra were then recorded at 3 min intervals until exciplex emission did not increase further (this usually occurred after 9 min). If an exciplex emission peak at ~480 nm was not seen within this time frame, recording was extended for up to 30 min.

Melting temperature studies

Optical melting curves of the complexes were obtained using a Varian Cary 1E UV-visible spectrophotometer using a 1 mL optical cell (pathlength 1.0 cm). Thermal denaturation (T_m) measurements, detected at 260 nm and accurate to ±0.1 °C, were performed at 2.5 μM component concentrations of the 1 : 1 : 1 complex in 80% TFE–Tris buffer (10 mM Tris, pH 8.3, 100 mM NaCl). Melting data were also obtained from fluorescence emission spectra: the sample was heated to 60 °C at 0.25 °C min^{-1}. Wavelengths used were as follows: pyrene LES, λ_{ext} 340nm, λ_{em} 376 nm; exciplex band λ_{ext} 350 nm, λ_{em} 480 nm). Recordings were made at 0.5 °C increments. The sample was cooled (0.13 °C per min) and emission spectra again recorded. The melting curve obtained during cooling was used determine T_m, calculated either as the point at half the curve height[41] or by derivative calculation[42,43] using the Cary software.

Acknowledgements

We are grateful for financial support to the BBSRC and DTI, to the Genetic Innovation Network, and to Dr Khalil Azar of Proligo, Paris, for support. Mr Abdul Gbaj is grateful to the Libyan Cultural Affairs Office (London) for financial support. We are also grateful to the University of Manchester and Dr Martino Picardo of University of Manchester Incubator Company Ltd (http://www.umic.co.uk).

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EXHIBIT C

RELATED PROCEEDINGS APPENDIX

1. *Phillips v. AWH Corp.*, 415 F.3d 1303, 75 USPQ2d 1321 (Fed. Cir. 2005) (*en banc*)
2. *Vitronics Corp. v. Conceptronic Inc.*, 90 F.3d 1576, 39 USPQ2d 1573, 1577 (Fed. Cir. 1996)
3. *Novartis Pharms. Corp. v. Abbott Labs.*, 375 F.3d 1328, 1334-35 (Fed. Cir. 2004)

▷

Briefs and Other Related Documents

United States Court of Appeals,
Federal Circuit.
Edward H. PHILLIPS, Plaintiff-Appellant,
v.
AWH CORPORATION, Hopeman Brothers, Inc., and Lofton Corporation, Defendants-Cross Appellants.
Nos. 03-1269, 03-1286.

July 12, 2005.

Background: Owner of patent for vandalism-resistant wall panels sued former distributor for infringement. The United States District Court for the District of Colorado, Marcia S. Krieger, J., granted summary judgment for former distributor, and owner appealed. The Court of Appeals, 363 F.3d 1207, affirmed.

Holding: On rehearing en banc, the Court of Appeals, Bryson, Circuit Judge, held that "baffles," called for in asserted claim, were not limited to non-perpendicular, projectile-deflecting structures disclosed in preferred embodiment.
Affirmed in part, reversed in part, dismissed in part, and remanded.

Lourie, Circuit Judge, concurred in part, dissenting in part, and filed opinion in which Pauline Newman, Circuit Judge, joined.

Mayer, Circuit Judge, dissented and filed opinion in which Pauline Newman, Circuit Judge, joined.

West Headnotes

[1] **Patents 101(8)**
291k101(8) Most Cited Cases
Limitation in patent claim for vandalism-resistant wall, calling for "means disposed inside shell for increasing its load bearing capacity" comprising "internal steel baffles" extending inwardly from steel shell walls, recited sufficient structure to avoid means-plus-function treatment. 35 U.S.C.A. § 112, par. 6.

[2] **Patents 165(2)**
291k165(2) Most Cited Cases
It is bedrock principle of patent law that claims of patent define invention to which patentee is entitled the right to exclude.

[3] **Patents 157(1)**
291k157(1) Most Cited Cases

[3] **Patents 161**
291k161 Most Cited Cases
Words of patent claim are generally given their ordinary and customary meaning, i.e., meaning that term would have to person of ordinary skill in the art in question on effective filing date of patent application.

[4] **Patents 161**
291k161 Most Cited Cases

[4] **Patents 167(1)**
291k167(1) Most Cited Cases
Person of ordinary skill in the art, through whose eyes patent claim is construed, is deemed to read claim term not only in context of particular claim in which disputed term appears, but in context of entire patent, including specification.

[5] **Patents 165(5)**
291k165(5) Most Cited Cases
Because claim terms are normally used consistently throughout patent, usage of term in one claim can often illuminate meaning of same term in other claims.

[6] **Patents 165(5)**
291k165(5) Most Cited Cases
Presence of dependent patent claim that adds particular limitation gives rise to presumption that limitation in question is not present in independent claim.

[7] **Patents 101(4)**
291k101(4) Most Cited Cases
Patent claims must be read in view of specification, of which they are part.

[8] **Patents 167(1)**
291k167(1) Most Cited Cases
Patent specification is always highly relevant to claim

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construction analysis; it is single best guide to meaning of disputed term, and is usually dispositive.

[9] Patents 162
291k162 Most Cited Cases

[9] Patents 167(1.1)
291k167(1.1) Most Cited Cases
When patent specification reveals special definition given to claim term by patentee that differs from meaning it would otherwise possess, inventor's lexicography governs.

[10] Patents 167(1.1)
291k167(1.1) Most Cited Cases
Where patent specification reveals intentional disclaimer, or disavowal, of claim scope by inventor, such revealed intention is dispositive.

[11] Patents 168(1)
291k168(1) Most Cited Cases
"Prosecution history" consists of complete record of proceedings before Patent and Trademark Office and includes prior art cited during examination of patent.

[12] Patents 168(2.1)
291k168(2.1) Most Cited Cases
Prosecution history can often inform meaning of patent claim language by demonstrating how inventor understood invention and whether inventor limited invention in course of prosecution, making claim scope narrower than it would otherwise be.

[13] Patents 159
291k159 Most Cited Cases
Although extrinsic evidence can shed useful light on relevant art, it is less significant than intrinsic record in determining legally operative meaning of patent claim language.

[14] Patents 159
291k159 Most Cited Cases
Dictionaries, though extrinsic evidence, are among tools that can assist patent-construing court in determining meaning of particular terminology to those of skill in the art of invention.

[15] Patents 159
291k159 Most Cited Cases
Extrinsic evidence in form of expert testimony can be useful to patent-construing court for variety of purposes, such as to provide background on technology at issue, to explain how invention works, to ensure that court's understanding of technical aspects of patent is consistent with that of person of skill in the art, or to establish that particular term in patent or prior art has particular meaning in pertinent field.

[16] Patents 159
291k159 Most Cited Cases
Conclusory, unsupported assertions by experts as to definition of patent claim term are not useful to construing court.

[17] Patents 159
291k159 Most Cited Cases
Court, when construing patent, should discount any expert testimony that is clearly at odds with claim construction mandated by claims themselves, written description, and prosecution history.

[18] Patents 159
291k159 Most Cited Cases
Extrinsic evidence may be useful to construing court, but it is unlikely to result in reliable interpretation of patent claim scope unless considered in context of intrinsic evidence.

[19] Patents 159
291k159 Most Cited Cases
It is permissible for patent-construing court, in its sound discretion, to admit and use extrinsic evidence; such evidence can help educate court regarding field of invention and can help court determine what person of ordinary skill in the art would understand claim terms to mean.

[20] Patents 159
291k159 Most Cited Cases
Although dictionaries or comparable sources are often useful to assist in understanding commonly understood meaning of patent claim terms, construing court's focus remains on understanding how person of ordinary skill in the art would understand claim terms.

[21] Patents 157(1)
291k157(1) Most Cited Cases
There is no magic formula or catechism for conducting patent claim construction; court is not barred from considering any particular sources or required to analyze sources in any specific sequence, so long as those sources are not used to contradict claim meaning that is unambiguous in light of intrinsic evidence.

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[22] Patents 101(3)
291k101(3) Most Cited Cases
Internal steel "baffles," called for in patent for vandalism-resistant wall panels, were not limited to non-perpendicular, projectile-deflecting structures disclosed in preferred embodiment; specification did not require that internal structures always be capable of performing that function.

[23] Patents 101(3)
291k101(3) Most Cited Cases
Fact that patent asserts that invention achieves several objectives does not require that each claim be construed as limited to structures that are capable of achieving all those objectives.

[24] Patents 157(2)
291k157(2) Most Cited Cases
Application of patent construction maxim, that claims should be so construed, if possible, as to sustain their validity, is limited to cases in which court concludes, after applying all available tools of claim construction, that claim is still ambiguous.

Patents 328(2)
291k328(2) Most Cited Cases
4,677,798. Construed.
***1305** Carl F. Manthei, Attorney at Law, of Boulder, Colorado, argued for plaintiff-appellant.

Mark W. Fischer, Faegre & Benson LLP, of Boulder, Colorado, argued for defendants-cross appellants. With him on the brief were Neal S. Cohen and Peter J. Kinsella. Of counsel on the brief were Maurice M. Klee, Law Office of Maurice M. Klee, Ph.D., Fairfield, Connecticut, and Kenneth C. Bass, III, Sterne, Kessler, Goldstein & Fox, of Washington, DC. Of counsel was Scott E. Holwick, Faegre & Benson LLP.

***1306** John M. Whealan, Solicitor, United States Patent and Trademark Office, of Alexandria, Virginia, argued for amicus curiae United States. With him on the brief were James A. Toupin, General Counsel; Cynthia C. Lynch; Linda Moncys Isacson; and Thomas W. Krause, Associate Solicitors. Of counsel on the brief were Peter D. Keisler, Assistant Attorney General; John J. Fargo, Director, Commercial Litigation Branch, and Anthony J. Steinmeyer, Attorney, Appellate Staff, Civil Division, United States Department of Justice, of Washington, DC; and William E. Kovacic, General Counsel, and Suzanne T. Michel, Chief Counsel for Intellectual Property, Federal Trade Commission, of Washington, DC.

Robert L. Baechtold, Fitzpatrick, Cella, Harper & Scinto, of New York, New York, for amici curiae Novartis Pharmaceuticals Corporation, et al. With him on the brief were Nicholas N. Kallas, Stevan J. Bosses, and Brian V. Slater.

Paul H. Berghoff, McDonnell Boehnen Hulbert & Berghoff LLP, of Chicago, Illinois, for amicus curiae Intellectual Property Owners Association. With him on the brief was Joshua R. Rich. Of counsel on the brief was J. Jeffery Hawley, President, Intellectual Property Owners Association, of Washington, DC. Of counsel was Herbert C. Wamsley, Intellectual Property Owners Association.

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R. Polk Wagner, University of Pennsylvania Law School, of Philadelphia, Pennsylvania, for amicus curiae Patent Law Professors R. Polk Wagner, et al.

Joshua D. Sarnoff, Washington College of Law, American University, of Washington, DC, for amici curiae Consumers Union, et al.

Laura M. Slenzak, Siemens Corporation, of Auburn Hills, Michigan, for amicus curiae The State Bar of Michigan, Intellectual Property Law Section, joined in the brief of the New York Intellectual Property Law Association.

Lea Hall Speed, Baker, Donelson, Bearman & Caldwell, of Memphis, Tennessee, for amicus curiae Tennessee Bar Association, joined in the brief of the New York Intellectual Property Law Association.

Before MICHEL, Chief Judge, NEWMAN, MAYER, LOURIE, CLEVENGER, RADER, SCHALL, BRYSON, GAJARSA, LINN, DYK, and PROST, Circuit Judges.

Opinion for the court filed by Circuit Judge BRYSON, in which Chief Judge MICHEL and Circuit Judges CLEVENGER, RADER, SCHALL, GAJARSA, LINN, DYK, and PROST join; and in which Circuit Judge LOURIE joins with respect to parts I, II, III, V, and VI; ***1309** and in which Circuit Judge PAULINE NEWMAN joins with respect to parts I, II, III, and V. Opinion concurring in part and dissenting in part filed by Circuit Judge LOURIE, in which Circuit Judge PAULINE NEWMAN joins. Dissenting opinion filed by Circuit Judge MAYER, in which Circuit Judge PAULINE NEWMAN joins.

BRYSON, Circuit Judge.

Edward H. Phillips invented modular, steel-shell panels that can be welded together to form vandalism-resistant walls. The panels are especially useful in building prisons because they are load-bearing and impact-resistant, while also insulating against fire and noise. Mr. Phillips obtained a patent on the invention, U.S. Patent No. 4,677,798 ("the '798 patent"), and he subsequently entered into an arrangement with AWH Corporation, Hopeman Brothers, Inc., and Lofton Corporation (collectively "AWH") to market and sell the panels. That arrangement ended in 1990. In 1991, however, Mr. Phillips received a sales brochure from AWH that suggested to him that AWH was continuing to use his trade secrets and patented technology without his consent. In a series of letters in 1991 and 1992, Mr. Phillips accused AWH of patent infringement and trade secret misappropriation. Correspondence between the parties regarding the matter ceased after that time.

In February 1997, Mr. Phillips brought suit in the United States District Court for the District of

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Colorado charging AWH with misappropriation of trade secrets and infringement of claims 1, 21, 22, 24, 25, and 26 of the '798 patent. *Phillips v. AWH Corp.*, No. 97-N-212 (D.Colo.). The district court dismissed the trade secret misappropriation claim as barred by Colorado's three-year statute of limitations.

With regard to the patent infringement issue, the district court focused on the language of claim 1, which recites "further means disposed inside the shell for increasing its load bearing capacity comprising internal steel baffles extending inwardly from the steel shell walls." The court interpreted that language as "a means ... for performing a specified function," subject to 35 U.S.C. § 112, paragraph 6, which provides that such a claim "shall be construed to cover the corresponding structure, material, or acts described in the specification and equivalents thereof." Looking to the specification of the '798 patent, the court noted that "every textual reference in the Specification and its diagrams show baffle deployment at an angle other than 90 to the wall faces" and that "placement of the baffles at such angles creates an intermediate interlocking, but not solid, internal barrier." The district court therefore ruled that, for purposes of the '798 patent, a baffle must "extend inward from the steel shell walls at an oblique or acute angle to the wall face" and must form part of an interlocking barrier in the interior of the wall module. Because Mr. Phillips could not prove infringement under that claim construction, the district court granted summary judgment of noninfringement.

Mr. Phillips appealed with respect to both the trade secret and patent infringement claims. A panel of this court affirmed on both issues. *Phillips v. AWH Corp.*, 363 F.3d 1207 (Fed.Cir.2004). As to the trade secret claim, the panel unanimously upheld the district court's ruling that the claim was barred by the applicable statute of limitations. *Id.* at 1215. As to the patent infringement claims, the panel was divided. The majority sustained the district court's summary judgment of noninfringement, although on different grounds. The dissenting judge would have reversed the summary judgment of noninfringement.

***1310** The panel first determined that because the asserted claims of the '798 patent contain a sufficient recitation of structure, the district court erred by construing the term "baffles" to invoke the "means-plus-function" claim format authorized by section 112, paragraph 6. *Id.* at 1212. Nonetheless, the panel concluded that the patent uses the term "baffles" in a restrictive manner. Based on the patent's written description, the panel held that the claim term "baffles" excludes structures that extend at a 90 degree angle from the walls. The panel noted that the specification repeatedly refers to the ability of the claimed baffles to deflect projectiles and that it describes the baffles as being "disposed at such angles that bullets which might penetrate the outer steel panels are deflected." '798 patent, col. 2, ll. 13-15; *see also id.* at col. 5, ll. 17-19 (baffles are "disposed at angles which tend to deflect the bullets"). In addition, the panel observed that nowhere in the patent is there any disclosure of a baffle projecting from the wall at a right angle and that baffles oriented at 90 degrees to the wall were found in the prior art. Based on "the specification's explicit descriptions," the panel concluded "that the patentee regarded his invention as panels providing impact or projectile resistance and that the baffles must be oriented at angles other than 90." *Phillips*, 363 F.3d at 1213. The panel added that the patent specification "is intended to support and inform the claims, and here it makes it unmistakably clear that the invention involves baffles angled at other than 90." *Id.* at 1214. The panel therefore upheld the district court's summary judgment of noninfringement.

The dissenting judge argued that the panel had improperly limited the claims to the particular embodiment of the invention disclosed in the specification, rather than adopting the "plain meaning" of the term "baffles." The dissenting judge noted that the parties had stipulated that "baffles" are a "means for obstructing, impeding, or checking the flow of something," and that the panel majority had agreed that the ordinary meaning of baffles is "something for deflecting, checking, or otherwise regulating flow." *Phillips*, 363 F.3d at 1216-17. In the dissent's view, nothing in the specification redefined the term "baffles" or constituted a disclaimer specifically limiting the term to less than the full scope of its ordinary meaning. Instead, the dissenting judge contended, the specification "merely identifies impact resistance as one of several objectives of the invention." *Id.* at 1217. In sum, the dissent concluded that "there is no reason to supplement the plain meaning of the claim language with a limitation from the preferred embodiment." *Id.* at 1218. Consequently, the dissenting judge argued that the court should have adopted the general purpose dictionary definition of the term baffle, i.e., "something for deflecting, checking, or otherwise regulating flow," *id.*, and therefore should have reversed the summary judgment of noninfringement.

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This court agreed to rehear the appeal en banc and vacated the judgment of the panel. *Phillips v. AWH Corp.*, 376 F.3d 1382 (Fed.Cir.2004). We now affirm the portion of the district court's judgment addressed to the trade secret misappropriation claims. However, we reverse the portion of the court's judgment addressed to the issue of infringement.

I

Claim 1 of the '798 patent is representative of the asserted claims with respect to the use of the term "baffles." It recites:

> Building modules adapted to fit together for construction of fire, sound and impact resistant security barriers and rooms for use in securing records and ***1311** persons, comprising in combination, an outer shell ..., sealant means ... and further means disposed inside the shell for increasing its load bearing capacity comprising internal steel baffles extending inwardly from the steel shell walls.

[1] As a preliminary matter, we agree with the panel that the term "baffles" is not means-plus-function language that invokes 35 U.S.C. § 112, paragraph 6. To be sure, the claim refers to "means disposed inside the shell for increasing its load bearing capacity," a formulation that would ordinarily be regarded as invoking the means-plus-function claim format. However, the claim specifically identifies "internal steel baffles" as structure that performs the recited function of increasing the shell's load-bearing capacity. In contrast to the "load bearing means" limitation, the reference to "baffles" does not use the word "means," and we have held that the absence of that term creates a rebuttable presumption that section 112, paragraph 6, does not apply. *See Personalized Media Communications, LLC v. Int'l Trade Comm'n*, 161 F.3d 696, 703-04 (Fed.Cir.1998).

Means-plus-function claiming applies only to purely functional limitations that do not provide the structure that performs the recited function. *See Watts v. XL Sys., Inc.*, 232 F.3d 877, 880-81 (Fed.Cir.2000). While the baffles in the '798 patent are clearly intended to perform several functions, the term "baffles" is nonetheless structural; it is not a purely functional placeholder in which structure is filled in by the specification. *See TurboCare Div. of Demag Delaval Turbomachinery Corp. v. Gen. Elec. Co.*, 264 F.3d 1111, 1121 (Fed.Cir.2001) (reasoning that nothing in the specification or prosecution history suggests that the patentee used the term "compressed spring" to denote any structure that is capable of performing the specified function); *Greenberg v. Ethicon Endo-Surgery, Inc.*, 91 F.3d 1580, 1583 (Fed.Cir.1996) (construing the term "detent mechanism" to refer to particular structure, even though the term has functional connotations). The claims and the specification unmistakably establish that the "steel baffles" refer to particular physical apparatus. The claim characterizes the baffles as "extend [ing] inwardly" from the steel shell walls, which plainly implies that the baffles are structures. The specification likewise makes clear that the term "steel baffles" refers to particular internal wall structures and is not simply a general description of any structure that will perform a particular function. *See, e.g.*, '798 patent, col. 4, ll. 25-26 ("the load bearing baffles 16 are optionally used with longer panels"); *id.*, col. 4, ll. 49-50 (opposing panels are "compressed between the flange 35 and the baffle 26"). Because the term "baffles" is not subject to section 112, paragraph 6, we agree with the panel that the district court erred by limiting the term to corresponding structures disclosed in the specification and their equivalents. Accordingly, we must determine the correct construction of the structural term "baffles," as used in the '798 patent.

II

The first paragraph of section 112 of the Patent Act, 35 U.S.C. § 112, states that the specification

> shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains ... to make and use the same

The second paragraph of section 112 provides that the specification

> shall conclude with one or more claims particularly pointing out and distinctly ***1312** claiming the subject matter which the applicant regards as his invention.

Those two paragraphs of section 112 frame the issue of claim interpretation for us. The second paragraph requires us to look to the language of the claims to determine what "the applicant regards as his invention." On the other hand, the first paragraph requires that the specification describe the invention set forth in the claims. The principal question that this case presents to us is the extent to which we should resort to and rely on a patent's specification in seeking to ascertain the proper scope of its claims.

This is hardly a new question. The role of the specification in claim construction has been an issue in patent law decisions in this country for nearly two centuries. We addressed the relationship between the

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specification and the claims at some length in our en banc opinion in *Markman v. Westview Instruments, Inc.*, 52 F.3d 967, 979-81 (Fed.Cir.1995) (en banc), *aff'd*, 517 U.S. 370, 116 S.Ct. 1384, 134 L.Ed.2d 577 (1996). We again summarized the applicable principles in *Vitronics Corp. v. Conceptronic, Inc.*, 90 F.3d 1576 (Fed.Cir.1996), and more recently in *Innova/Pure Water, Inc. v. Safari Water Filtration Systems, Inc.*, 381 F.3d 1111 (Fed.Cir.2004). What we said in those cases bears restating, for the basic principles of claim construction outlined there are still applicable, and we reaffirm them today. We have also previously considered the use of dictionaries in claim construction. What we have said in that regard requires clarification.

A

[2] It is a "bedrock principle" of patent law that "the claims of a patent define the invention to which the patentee is entitled the right to exclude." *Innova*, 381 F.3d at 1115; *see also Vitronics*, 90 F.3d at 1582 ("we look to the words of the claims themselves ... to define the scope of the patented invention"); *Markman*, 52 F.3d at 980 ("The written description part of the specification itself does not delimit the right to exclude. That is the function and purpose of claims."). That principle has been recognized since at least 1836, when Congress first required that the specification include a portion in which the inventor "shall particularly specify and point out the part, improvement, or combination, which he claims as his own invention or discovery." Act of July 4, 1836, ch. 357, § 6, 5 Stat. 117, 119. In the following years, the Supreme Court made clear that the claims are "of primary importance, in the effort to ascertain precisely what it is that is patented." *Merrill v. Yeomans*, 94 U.S. 568, 570, 24 L.Ed. 235 (1876). Because the patentee is required to "define precisely what his invention is," the Court explained, it is "unjust to the public, as well as an evasion of the law, to construe it in a manner different from the plain import of its terms." *White v. Dunbar*, 119 U.S. 47, 52, 7 S.Ct. 72, 30 L.Ed. 303 (1886); *see also Cont'l Paper Bag Co. v. E. Paper Bag Co.*, 210 U.S. 405, 419, 28 S.Ct. 748, 52 L.Ed. 1122 (1908) ("the claims measure the invention"); *McCarty v. Lehigh Valley R.R. Co.*, 160 U.S. 110, 116, 16 S.Ct. 240, 40 L.Ed. 358 (1895) ("if we once begin to include elements not mentioned in the claim, in order to limit such claim ..., we should never know where to stop"); *Aro Mfg. Co. v. Convertible Top Replacement Co.*, 365 U.S. 336, 339, 81 S.Ct. 599, 5 L.Ed.2d 592 (1961) ("the claims made in the patent are the sole measure of the grant").

[3] We have frequently stated that the words of a claim "are generally given their ordinary and customary meaning." *Vitronics*, 90 F.3d at 1582; *see also Toro Co. v. White Consol. Indus., Inc.*, 199 F.3d 1295, 1299 (Fed.Cir.1999); ***1313***Renishaw PLC v. Marposs Societa' per Azioni*, 158 F.3d 1243, 1249 (Fed.Cir.1998). We have made clear, moreover, that the ordinary and customary meaning of a claim term is the meaning that the term would have to a person of ordinary skill in the art in question at the time of the invention, i.e., as of the effective filing date of the patent application. *See Innova*, 381 F.3d at 1116 ("A court construing a patent claim seeks to accord a claim the meaning it would have to a person of ordinary skill in the art at the time of the invention."); *Home Diagnostics, Inc. v. LifeScan, Inc.*, 381 F.3d 1352, 1358 (Fed.Cir.2004) ("customary meaning" refers to the "customary meaning in [the] art field"); *Ferguson Beauregard/Logic Controls v. Mega Sys., LLC*, 350 F.3d 1327, 1338 (Fed.Cir.2003) (claim terms "are examined through the viewing glass of a person skilled in the art"); *see also PC Connector Solutions LLC v. SmartDisk Corp.*, 406 F.3d 1359, 1363 (Fed.Cir.2005) (meaning of claim "must be interpreted as of [the] effective filing date" of the patent application); *Schering Corp. v. Amgen Inc.*, 222 F.3d 1347, 1353 (Fed.Cir.2000) (same).

The inquiry into how a person of ordinary skill in the art understands a claim term provides an objective baseline from which to begin claim interpretation. *See Innova*, 381 F.3d at 1116. That starting point is based on the well-settled understanding that inventors are typically persons skilled in the field of the invention and that patents are addressed to and intended to be read by others of skill in the pertinent art. *See Verve, LLC v. Crane Cams, Inc.*, 311 F.3d 1116, 1119 (Fed.Cir.2002) (patent documents are meant to be "a concise statement for persons in the field"); *In re Nelson*, 47 C.C.P.A. 1031, 280 F.2d 172, 181 (1960) ("The descriptions in patents are not addressed to the public generally, to lawyers or to judges, but, as section 112 says, to those skilled in the art to which the invention pertains or with which it is most nearly connected.").

[4] Importantly, the person of ordinary skill in the art is deemed to read the claim term not only in the context of the particular claim in which the disputed term appears, but in the context of the entire patent, including the specification. This court explained that point well in *Multiform Desiccants, Inc. v. Medzam, Ltd.*, 133 F.3d 1473, 1477 (Fed.Cir.1998):

> It is the person of ordinary skill in the field of the invention through whose eyes the claims are

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> construed. Such person is deemed to read the words used in the patent documents with an understanding of their meaning in the field, and to have knowledge of any special meaning and usage in the field. The inventor's words that are used to describe the invention--the inventor's lexicography--must be understood and interpreted by the court as they would be understood and interpreted by a person in that field of technology. Thus the court starts the decisionmaking process by reviewing the same resources as would that person, *viz.*, the patent specification and the prosecution history.

See also Medrad, Inc. v. MRI Devices Corp., 401 F.3d 1313, 1319 (Fed.Cir.2005) ("We cannot look at the ordinary meaning of the term ... in a vacuum. Rather, we must look at the ordinary meaning in the context of the written description and the prosecution history."); V-Formation, Inc. v. Benetton Group SpA, 401 F.3d 1307, 1310 (Fed.Cir.2005) (intrinsic record "usually provides the technological and temporal context to enable the court to ascertain the meaning of the claim to one of ordinary skill in the art at the time of the invention"); ***1314***Unitherm Food Sys., Inc. v. Swift-Eckrich, Inc.*, 375 F.3d 1341, 1351 (Fed.Cir.2004) (proper definition is the "definition that one of ordinary skill in the art could ascertain from the intrinsic evidence in the record").

B

In some cases, the ordinary meaning of claim language as understood by a person of skill in the art may be readily apparent even to lay judges, and claim construction in such cases involves little more than the application of the widely accepted meaning of commonly understood words. *See* Brown v. 3M, 265 F.3d 1349, 1352 (Fed.Cir.2001) (holding that the claims did "not require elaborate interpretation"). In such circumstances, general purpose dictionaries may be helpful. In many cases that give rise to litigation, however, determining the ordinary and customary meaning of the claim requires examination of terms that have a particular meaning in a field of art. Because the meaning of a claim term as understood by persons of skill in the art is often not immediately apparent, and because patentees frequently use terms idiosyncratically, the court looks to "those sources available to the public that show what a person of skill in the art would have understood disputed claim language to mean." *Innova*, 381 F.3d at 1116. Those sources include "the words of the claims themselves, the remainder of the specification, the prosecution history, and extrinsic evidence concerning relevant scientific principles, the meaning of technical terms, and the state of the art." *Id.*; *see also* Gemstar-TV Guide Int'l, Inc. v. Int'l Trade Comm'n, 383 F.3d 1352, 1364 (Fed.Cir.2004); *Vitronics*, 90 F.3d at 1582-83; *Markman*, 52 F.3d at 979-80.

1

Quite apart from the written description and the prosecution history, the claims themselves provide substantial guidance as to the meaning of particular claim terms. *See* Vitronics, 90 F.3d at 1582; *see also* ACTV, Inc. v. Walt Disney Co., 346 F.3d 1082, 1088 (Fed.Cir.2003) ("the context of the surrounding words of the claim also must be considered in determining the ordinary and customary meaning of those terms").

To begin with, the context in which a term is used in the asserted claim can be highly instructive. To take a simple example, the claim in this case refers to "steel baffles," which strongly implies that the term "baffles" does not inherently mean objects made of steel. This court's cases provide numerous similar examples in which the use of a term within the claim provides a firm basis for construing the term. *See, e.g.*, Mars, Inc. v. H.J. Heinz Co., 377 F.3d 1369, 1374 (Fed.Cir.2004) (claim term "ingredients" construed in light of the use of the term "mixture" in the same claim phrase); Process Control Corp. v. HydReclaim Corp., 190 F.3d 1350, 1356 (Fed.Cir.1999) (claim term "discharge rate" construed in light of the use of the same term in another limitation of the same claim).

[5][6] Other claims of the patent in question, both asserted and unasserted, can also be valuable sources of enlightenment as to the meaning of a claim term. *Vitronics*, 90 F.3d at 1582. Because claim terms are normally used consistently throughout the patent, the usage of a term in one claim can often illuminate the meaning of the same term in other claims. *See* Rexnord Corp. v. Laitram Corp., 274 F.3d 1336, 1342 (Fed.Cir.2001); CVI/Beta Ventures, Inc. v. Tura LP, 112 F.3d 1146, 1159 (Fed.Cir.1997). Differences among claims can also be a useful guide in understanding the meaning of particular claim terms. *See* Laitram Corp. v. Rexnord, Inc., 939 F.2d 1533, 1538 (Fed.Cir.1991). For example, ***1315** the presence of a dependent claim that adds a particular limitation gives rise to a presumption that the limitation in question is not present in the independent claim. *See* Liebel-Flarsheim Co. v. Medrad, Inc., 358 F.3d 898, 910 (Fed.Cir.2004).

2

[7][8] The claims, of course, do not stand alone. Rather, they are part of "a fully integrated written instrument," *Markman*, 52 F.3d at 978, consisting

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principally of a specification that concludes with the claims. For that reason, claims "must be read in view of the specification, of which they are a part." *Id.* at 979. As we stated in *Vitronics*, the specification "is always highly relevant to the claim construction analysis. Usually, it is dispositive; it is the single best guide to the meaning of a disputed term." 90 F.3d at 1582.

This court and its predecessors have long emphasized the importance of the specification in claim construction. In *Autogiro Co. of America v. United States*, 181 Ct.Cl. 55, 384 F.2d 391, 397-98 (1967), the Court of Claims characterized the specification as "a concordance for the claims," based on the statutory requirement that the specification "describe the manner and process of making and using" the patented invention. The Court of Customs and Patent Appeals made a similar point. *See In re Fout*, 675 F.2d 297, 300 (CCPA 1982) ("Claims must always be read in light of the specification. Here, the specification makes plain what the appellants did and did not invent").

Shortly after the creation of this court, Judge Rich wrote that "[t]he descriptive part of the specification aids in ascertaining the scope and meaning of the claims inasmuch as the words of the claims must be based on the description. The specification is, thus, the primary basis for construing the claims." *Standard Oil Co. v. Am. Cyanamid Co.*, 774 F.2d 448, 452 (Fed.Cir.1985). On numerous occasions since then, we have reaffirmed that point, stating that "[t]he best source for understanding a technical term is the specification from which it arose, informed, as needed, by the prosecution history." *Multiform Desiccants*, 133 F.3d at 1478; *Metabolite Labs., Inc. v. Lab. Corp. of Am. Holdings*, 370 F.3d 1354, 1360 (Fed.Cir.2004) ("In most cases, the best source for discerning the proper context of claim terms is the patent specification wherein the patent applicant describes the invention."); *see also, e.g., Kinik Co. v. Int'l Trade Comm'n*, 362 F.3d 1359, 1365 (Fed.Cir.2004) ("The words of patent claims have the meaning and scope with which they are used in the specification and the prosecution history."); *Moba, B.V. v. Diamond Automation, Inc.*, 325 F.3d 1306, 1315 (Fed.Cir.2003) ("[T]he best indicator of claim meaning is its usage in context as understood by one of skill in the art at the time of invention.").

That principle has a long pedigree in Supreme Court decisions as well. *See Hogg v. Emerson*, 47 U.S. (6 How.) 437, 482, 12 L.Ed. 505 (1848) (the specification is a "component part of the patent" and "is as much to be considered with the [letters patent] in construing them, as any paper referred to in a deed or other contract"); *Bates v. Coe*, 98 U.S. 31, 38, 25 L.Ed. 68 (1878) ("in case of doubt or ambiguity it is proper in all cases to refer back to the descriptive portions of the specification to aid in solving the doubt or in ascertaining the true intent and meaning of the language employed in the claims"); *White v. Dunbar*, 119 U.S. 47, 51, 7 S.Ct. 72, 30 L.Ed. 303 (1886) (specification is appropriately resorted to "for the purpose of better understanding the meaning of the claim"); ***1316** *Schriber-Schroth Co. v. Cleveland Trust Co.*, 311 U.S. 211, 217, 61 S.Ct. 235, 85 L.Ed. 132 (1940) ("The claims of a patent are always to be read or interpreted in light of its specifications."); *United States v. Adams*, 383 U.S. 39, 49, 86 S.Ct. 708, 15 L.Ed.2d 572 (1966) ("[I]t is fundamental that claims are to be construed in the light of the specifications and both are to be read with a view to ascertaining the invention.").

The importance of the specification in claim construction derives from its statutory role. The close kinship between the written description and the claims is enforced by the statutory requirement that the specification describe the claimed invention in "full, clear, concise, and exact terms." 35 U.S.C. § 112, para. 1; *see Netword, LLC v. Centraal Corp.*, 242 F.3d 1347, 1352 (Fed.Cir.2001) ("The claims are directed to the invention that is described in the specification; they do not have meaning removed from the context from which they arose."); *see also Markman v. Westview Instruments, Inc.*, 517 U.S. 370, 389, 116 S.Ct. 1384, 134 L.Ed.2d 577 (1996) ("[A claim] term can be defined only in a way that comports with the instrument as a whole."). In light of the statutory directive that the inventor provide a "full" and "exact" description of the claimed invention, the specification necessarily informs the proper construction of the claims. *See Merck & Co. v. Teva Pharms. USA, Inc.*, 347 F.3d 1367, 1371 (Fed.Cir.2003) ("A fundamental rule of claim construction is that terms in a patent document are construed with the meaning with which they are presented in the patent document. Thus claims must be construed so as to be consistent with the specification, of which they are a part.") (citations omitted). In *Renishaw*, this court summarized that point succinctly:

> Ultimately, the interpretation to be given a term can only be determined and confirmed with a full understanding of what the inventors actually invented and intended to envelop with the claim. The construction that stays true to the claim language and most naturally aligns with the

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patent's description of the invention will be, in the end, the correct construction.
158 F.3d at 1250 (citations omitted).

[9][10] Consistent with that general principle, our cases recognize that the specification may reveal a special definition given to a claim term by the patentee that differs from the meaning it would otherwise possess. In such cases, the inventor's lexicography governs. *See CCS Fitness, Inc. v. Brunswick Corp.*, 288 F.3d 1359, 1366 (Fed.Cir.2002). In other cases, the specification may reveal an intentional disclaimer, or disavowal, of claim scope by the inventor. In that instance as well, the inventor has dictated the correct claim scope, and the inventor's intention, as expressed in the specification, is regarded as dispositive. *See SciMed Life Sys., Inc. v. Advanced Cardiovascular Sys., Inc.*, 242 F.3d 1337, 1343-44 (Fed.Cir.2001).

The pertinence of the specification to claim construction is reinforced by the manner in which a patent is issued. The Patent and Trademark Office ("PTO") determines the scope of claims in patent applications not solely on the basis of the claim language, but upon giving claims their broadest reasonable construction "in light of the specification as it would be interpreted by one of ordinary skill in the art." *In re Am. Acad. of Sci. Tech. Ctr.*, 367 F.3d 1359, 1364 (Fed.Cir.2004). Indeed, the rules of the PTO require that application claims must "conform to the invention as set forth in the remainder of the specification and the terms and phrases used in the claims must find clear support or antecedent basis in the description ***1317** so that the meaning of the terms in the claims may be ascertainable by reference to the description." 37 C.F.R. § 1.75(d)(1). It is therefore entirely appropriate for a court, when conducting claim construction, to rely heavily on the written description for guidance as to the meaning of the claims.

3

[11][12] In addition to consulting the specification, we have held that a court "should also consider the patent's prosecution history, if it is in evidence." *Markman*, 52 F.3d at 980; *see also Graham v. John Deere Co.*, 383 U.S. 1, 33, 86 S.Ct. 684, 15 L.Ed.2d 545 (1966) ("[A]n invention is construed not only in the light of the claims, but also with reference to the file wrapper or prosecution history in the Patent Office."). The prosecution history, which we have designated as part of the "intrinsic evidence," consists of the complete record of the proceedings before the PTO and includes the prior art cited during the examination of the patent. *Autogiro*, 384 F.2d at 399. Like the specification, the prosecution history provides evidence of how the PTO and the inventor understood the patent. *See Lemelson v. Gen. Mills, Inc.*, 968 F.2d 1202, 1206 (Fed.Cir.1992). Furthermore, like the specification, the prosecution history was created by the patentee in attempting to explain and obtain the patent. Yet because the prosecution history represents an ongoing negotiation between the PTO and the applicant, rather than the final product of that negotiation, it often lacks the clarity of the specification and thus is less useful for claim construction purposes. *See Inverness Med. Switz. GmbH v. Warner Lambert Co.*, 309 F.3d 1373, 1380-82 (Fed.Cir.2002) (the ambiguity of the prosecution history made it less relevant to claim construction); *Athletic Alternatives, Inc. v. Prince Mfg., Inc.*, 73 F.3d 1573, 1580 (Fed.Cir.1996) (the ambiguity of the prosecution history made it "unhelpful as an interpretive resource" for claim construction). Nonetheless, the prosecution history can often inform the meaning of the claim language by demonstrating how the inventor understood the invention and whether the inventor limited the invention in the course of prosecution, making the claim scope narrower than it would otherwise be. *Vitronics*, 90 F.3d at 1582-83; *see also Chimie v. PPG Indus., Inc.*, 402 F.3d 1371, 1384 (Fed.Cir.2005) ("The purpose of consulting the prosecution history in construing a claim is to 'exclude any interpretation that was disclaimed during prosecution.' "), *quoting ZMI Corp. v. Cardiac Resuscitator Corp.*, 844 F.2d 1576, 1580 (Fed.Cir.1988); *Southwall Techs., Inc. v. Cardinal IG Co.*, 54 F.3d 1570, 1576 (Fed.Cir.1995).

C

[13] Although we have emphasized the importance of intrinsic evidence in claim construction, we have also authorized district courts to rely on extrinsic evidence, which "consists of all evidence external to the patent and prosecution history, including expert and inventor testimony, dictionaries, and learned treatises." *Markman*, 52 F.3d at 980, *citing Seymour v. Osborne*, 78 U.S. (11 Wall.) 516, 546, 20 L.Ed. 33 (1870); *see also Vitronics*, 90 F.3d at 1583. However, while extrinsic evidence "can shed useful light on the relevant art," we have explained that it is "less significant than the intrinsic record in determining 'the legally operative meaning of claim language.' " *C.R. Bard, Inc. v. U.S. Surgical Corp.*, 388 F.3d 858, 862 (Fed.Cir.2004), *quoting Vanderlande Indus. Nederland BV v. Int'l Trade Comm'n*, 366 F.3d 1311, 1318 (Fed.Cir.2004); *see also Astrazeneca AB v. Mutual Pharm. Co.*, 384 F.3d

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1333, 1337 (Fed.Cir.2004).

***1318** [14] Within the class of extrinsic evidence, the court has observed that dictionaries and treatises can be useful in claim construction. *See Renishaw*, 158 F.3d at 1250; *Rexnord*, 274 F.3d at 1344. We have especially noted the help that technical dictionaries may provide to a court "to better understand the underlying technology" and the way in which one of skill in the art might use the claim terms. *Vitronics*, 90 F.3d at 1584 n. 6. Because dictionaries, and especially technical dictionaries, endeavor to collect the accepted meanings of terms used in various fields of science and technology, those resources have been properly recognized as among the many tools that can assist the court in determining the meaning of particular terminology to those of skill in the art of the invention. *See Teleflex, Inc. v. Ficosa N. Am. Corp.*, 299 F.3d 1313, 1325 (Fed.Cir.2002). Such evidence, we have held, may be considered if the court deems it helpful in determining "the true meaning of language used in the patent claims." *Markman*, 52 F.3d at 980.

[15][16][17] We have also held that extrinsic evidence in the form of expert testimony can be useful to a court for a variety of purposes, such as to provide background on the technology at issue, to explain how an invention works, to ensure that the court's understanding of the technical aspects of the patent is consistent with that of a person of skill in the art, or to establish that a particular term in the patent or the prior art has a particular meaning in the pertinent field. *See Pitney Bowes, Inc. v. Hewlett-Packard Co.*, 182 F.3d 1298, 1308-09 (Fed.Cir.1999); *Key Pharms. v. Hercon Labs. Corp.*, 161 F.3d 709, 716 (Fed.Cir.1998). However, conclusory, unsupported assertions by experts as to the definition of a claim term are not useful to a court. Similarly, a court should discount any expert testimony "that is clearly at odds with the claim construction mandated by the claims themselves, the written description, and the prosecution history, in other words, with the written record of the patent." *Key Pharms.*, 161 F.3d at 716.

We have viewed extrinsic evidence in general as less reliable than the patent and its prosecution history in determining how to read claim terms, for several reasons. First, extrinsic evidence by definition is not part of the patent and does not have the specification's virtue of being created at the time of patent prosecution for the purpose of explaining the patent's scope and meaning. Second, while claims are construed as they would be understood by a hypothetical person of skill in the art, extrinsic publications may not be written by or for skilled artisans and therefore may not reflect the understanding of a skilled artisan in the field of the patent. Third, extrinsic evidence consisting of expert reports and testimony is generated at the time of and for the purpose of litigation and thus can suffer from bias that is not present in intrinsic evidence. The effect of that bias can be exacerbated if the expert is not one of skill in the relevant art or if the expert's opinion is offered in a form that is not subject to cross-examination. *See Senmed, Inc. v. Richard-Allan Med. Indus., Inc.*, 888 F.2d 815, 819 n. 8 (Fed.Cir.1989). Fourth, there is a virtually unbounded universe of potential extrinsic evidence of some marginal relevance that could be brought to bear on any claim construction question. In the course of litigation, each party will naturally choose the pieces of extrinsic evidence most favorable to its cause, leaving the court with the considerable task of filtering the useful extrinsic evidence from the fluff. *See Daubert v. Merrell Dow Pharms., Inc.*, 509 U.S. 579, 595, 113 S.Ct. 2786, 125 L.Ed.2d 469 (1993) ("Expert evidence can be both powerful and quite misleading because of the difficulty in evaluating it."). Finally, ***1319** undue reliance on extrinsic evidence poses the risk that it will be used to change the meaning of claims in derogation of the "indisputable public records consisting of the claims, the specification and the prosecution history," thereby undermining the public notice function of patents. *Southwall Techs.*, 54 F.3d at 1578.

[18][19] In sum, extrinsic evidence may be useful to the court, but it is unlikely to result in a reliable interpretation of patent claim scope unless considered in the context of the intrinsic evidence. Nonetheless, because extrinsic evidence can help educate the court regarding the field of the invention and can help the court determine what a person of ordinary skill in the art would understand claim terms to mean, it is permissible for the district court in its sound discretion to admit and use such evidence. In exercising that discretion, and in weighing all the evidence bearing on claim construction, the court should keep in mind the flaws inherent in each type of evidence and assess that evidence accordingly.

III

Although the principles outlined above have been articulated on numerous occasions, some of this court's cases have suggested a somewhat different approach to claim construction, in which the court has given greater emphasis to dictionary definitions of claim terms and has assigned a less prominent role

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to the specification and the prosecution history. The leading case in this line is *Texas Digital Systems, Inc. v. Telegenix, Inc.*, 308 F.3d 1193 (Fed.Cir.2002).

A

In *Texas Digital*, the court noted that "dictionaries, encyclopedias and treatises are particularly useful resources to assist the court in determining the ordinary and customary meanings of claim terms." 308 F.3d at 1202. Those texts, the court explained, are "objective resources that serve as reliable sources of information on the established meanings that would have been attributed to the terms of the claims by those of skill in the art," and they "deserve no less fealty in the context of claim construction" than in any other area of law. *Id.* at 1203. The court added that because words often have multiple dictionary meanings, the intrinsic record must be consulted to determine which of the different possible dictionary meanings is most consistent with the use of the term in question by the inventor. If more than one dictionary definition is consistent with the use of the words in the intrinsic record, the court stated, "the claim terms may be construed to encompass all such consistent meanings." *Id.*

The *Texas Digital* court further explained that the patent's specification and prosecution history must be consulted to determine if the patentee has used "the words [of the claim] in a manner clearly inconsistent with the ordinary meaning reflected, for example, in a dictionary definition." 308 F.3d at 1204. The court identified two circumstances in which such an inconsistency may be found. First, the court stated, "the presumption in favor of a dictionary definition will be overcome where the patentee, acting as his or her own lexicographer, has clearly set forth an explicit definition of the term different from its ordinary meaning." *Id.* Second, "the presumption also will be rebutted if the inventor has disavowed or disclaimed scope of coverage, by using words or expressions of manifest exclusion or restriction, representing a clear disavowal of claim scope." *Id.*

The *Texas Digital* court explained that it advanced the methodology set forth in ***1320** that opinion in an effort to combat what this court has termed "one of the cardinal sins of patent law--reading a limitation from the written description into the claims," *SciMed Life Sys.*, 242 F.3d at 1340. The court concluded that it is improper to consult "the written description and prosecution history as a threshold step in the claim construction process, before any effort is made to discern the ordinary and customary meanings attributed to the words themselves." *Texas Digital*, 308 F.3d at 1204. To do so, the court reasoned, "invites a violation of our precedent counseling against importing limitations into the claims." *Id.* Summarizing its analysis, the *Texas Digital* court stated:

> By examining relevant dictionaries, encyclopedias, and treatises to ascertain possible meanings that would have been attributed to the words of the claims by those skilled in the art, and by further utilizing the intrinsic record to select from those possible meanings the one or ones most consistent with the use of the words by the inventor, the full breadth of the limitations intended by the inventor will be more accurately determined and the improper importation of unintended limitations from the written description into the claims will be more easily avoided.

Id. at 1205.

B

Although the concern expressed by the court in *Texas Digital* was valid, the methodology it adopted placed too much reliance on extrinsic sources such as dictionaries, treatises, and encyclopedias and too little on intrinsic sources, in particular the specification and prosecution history. While the court noted that the specification must be consulted in every case, it suggested a methodology for claim interpretation in which the specification should be consulted only after a determination is made, whether based on a dictionary, treatise, or other source, as to the ordinary meaning or meanings of the claim term in dispute. Even then, recourse to the specification is limited to determining whether the specification excludes one of the meanings derived from the dictionary, whether the presumption in favor of the dictionary definition of the claim term has been overcome by "an explicit definition of the term different from its ordinary meaning," or whether the inventor "has disavowed or disclaimed scope of coverage, by using words or expressions of manifest exclusion or restriction, representing a clear disavowal of claim scope." 308 F.3d at 1204. In effect, the *Texas Digital* approach limits the role of the specification in claim construction to serving as a check on the dictionary meaning of a claim term if the specification requires the court to conclude that fewer than all the dictionary definitions apply, or if the specification contains a sufficiently specific alternative definition or disavowal. *See, e.g.*, *Texas Digital*, 308 F.3d at 1202 ("unless compelled otherwise, a court will give a claim term the full range of its ordinary meaning"); *Nystrom v. TREX Co.*, 374 F.3d 1105, 1111-13 (Fed.Cir.2004) (ascertaining the "full range" of the ordinary meaning

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of the term "board" through a collection of dictionary definitions, and stating that those candidate definitions should be removed from consideration only if they were "disclaimed" in the written description or prosecution history); *Inverness Med. Switz.*, 309 F.3d at 1379 (claim should be construed to encompass multiple dictionary meanings unless "the specification or prosecution history clearly demonstrates that only one of the multiple meanings was intended"). That approach, in our view, improperly restricts the role of the specification in claim construction.

Assigning such a limited role to the specification, and in particular requiring ***1321** that any definition of claim language in the specification be express, is inconsistent with our rulings that the specification is "the single best guide to the meaning of a disputed term," and that the specification "acts as a dictionary when it expressly defines terms used in the claims or when it defines terms by implication." *Vitronics*, 90 F.3d at 1582; *Irdeto Access, Inc. v. Echostar Satellite Corp.*, 383 F.3d 1295, 1300 (Fed.Cir.2004) ("Even when guidance is not provided in explicit definitional format, the specification may define claim terms by implication such that the meaning may be found in or ascertained by a reading of the patent documents.") (citations omitted); *Novartis Pharms. Corp. v. Abbott Labs.*, 375 F.3d 1328, 1334-35 (Fed.Cir.2004) (same); *Bell Atl. Network Servs., Inc. v. Covad Communications Group, Inc.*, 262 F.3d 1258, 1268 (Fed.Cir.2001) ("[A] claim term may be clearly redefined without an explicit statement of redefinition.").

The main problem with elevating the dictionary to such prominence is that it focuses the inquiry on the abstract meaning of words rather than on the meaning of claim terms within the context of the patent. Properly viewed, the "ordinary meaning" of a claim term is its meaning to the ordinary artisan after reading the entire patent. Yet heavy reliance on the dictionary divorced from the intrinsic evidence risks transforming the meaning of the claim term to the artisan into the meaning of the term in the abstract, out of its particular context, which is the specification. The patent system is based on the proposition that claims cover only the invented subject matter. As the Supreme Court has stated, "[i]t seems to us that nothing can be more just and fair, both to the patentee and the public, than that the former should understand, and correctly describe, just what he has invented, and for what he claims a patent." *Merrill v. Yeomans*, 94 U.S. at 573-74. The use of a dictionary definition can conflict with that directive because the patent applicant did not create the dictionary to describe the invention. Thus, there may be a disconnect between the patentee's responsibility to describe and claim his invention, and the dictionary editors' objective of aggregating all possible definitions for particular words.

Although the *Texas Digital* line of cases permit the dictionary definition to be narrowed in some circumstances even when there is not an explicit disclaimer or redefinition in the specification, too often that line of cases has been improperly relied upon to condone the adoption of a dictionary definition entirely divorced from the context of the written description. The problem is that if the district court starts with the broad dictionary definition in every case and fails to fully appreciate how the specification implicitly limits that definition, the error will systematically cause the construction of the claim to be unduly expansive. The risk of systematic overbreadth is greatly reduced if the court instead focuses at the outset on how the patentee used the claim term in the claims, specification, and prosecution history, rather than starting with a broad definition and whittling it down.

Dictionaries, by their nature, provide an expansive array of definitions. General dictionaries, in particular, strive to collect all uses of particular words, from the common to the obscure. By design, general dictionaries collect the definitions of a term as used not only in a particular art field, but in many different settings. In such circumstances, it is inevitable that the multiple dictionary definitions for a term will extend beyond the "construction of the patent [that] is confirmed by the avowed understanding of the patentee, expressed by him, or on his behalf, when his application ***1322** for the original patent was pending." *Goodyear Dental Vulcanite Co. v. Davis*, 102 U.S. 222, 227, 26 L.Ed. 149 (1880). Thus, the use of the dictionary may extend patent protection beyond what should properly be afforded by the inventor's patent. *See Smith v. Snow*, 294 U.S. 1, 14, 55 S.Ct. 279, 79 L.Ed. 721 (1935) ("if the claim were fairly susceptible of two constructions, that should be adopted which will secure to the patentee *his actual invention* ") (emphasis added). For that reason, we have stated that "a general-usage dictionary cannot overcome art-specific evidence of the meaning" of a claim term. *Vanderlande Indus. Nederland*, 366 F.3d at 1321; *see also Renishaw*, 158 F.3d at 1250, *quoting Liebscher v. Boothroyd*, 46 C.C.P.A. 701, 258 F.2d 948, 951 (CCPA 1958) ("Indiscriminate reliance on definitions found in dictionaries can often produce

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absurd results.... One need not arbitrarily pick and choose from the various accepted definitions of a word to decide which meaning was intended as the word is used in a given claim. The subject matter, the context, etc., will more often than not lead to the correct conclusion.").

Even technical dictionaries or treatises, under certain circumstances, may suffer from some of these deficiencies. There is no guarantee that a term is used in the same way in a treatise as it would be by the patentee. In fact, discrepancies between the patent and treatises are apt to be common because the patent by its nature describes something novel. *See* Autogiro, 384 F.2d at 397 ("Often the invention is novel and words do not exist to describe it. The dictionary does not always keep abreast of the inventor. It cannot.").

Moreover, different dictionaries may contain somewhat different sets of definitions for the same words. A claim should not rise or fall based upon the preferences of a particular dictionary editor, or the court's independent decision, uninformed by the specification, to rely on one dictionary rather than another. Finally, the authors of dictionaries or treatises may simplify ideas to communicate them most effectively to the public and may thus choose a meaning that is not pertinent to the understanding of particular claim language. *See generally* Ellen P. Aprill, *The Law of the Word: Dictionary Shopping in the Supreme Court*, 30 Ariz. St. L.J. 275, 293-314 (1998). The resulting definitions therefore do not necessarily reflect the inventor's goal of distinctly setting forth his invention as a person of ordinary skill in that particular art would understand it.

[20] As we have noted above, however, we do not intend to preclude the appropriate use of dictionaries. Dictionaries or comparable sources are often useful to assist in understanding the commonly understood meaning of words and have been used both by our court and the Supreme Court in claim interpretation. *See Exhibit Supply Co. v. Ace Patents Corp.*, 315 U.S. 126, 134, 62 S.Ct. 513, 86 L.Ed. 736 (1942) (relying on dictionaries to construe the claim term "embedded"); *Weber Elec. Co. v. E.H. Freeman Elec. Co.*, 256 U.S. 668, 678, 41 S.Ct. 600, 65 L.Ed. 1162 (1921) (approving circuit court's use of dictionary definitions to define claim terms); *Renishaw*, 158 F.3d at 1247-53 (approving the use of dictionaries with proper respect for the role of intrinsic evidence). A dictionary definition has the value of being an unbiased source "accessible to the public in advance of litigation." *Vitronics*, 90 F.3d at 1585. As we said in *Vitronics*, judges are free to consult dictionaries and technical treatises

> at any time in order to better understand the underlying technology and may also rely on dictionary definitions when construing claim terms, so long as the dictionary definition does not contradict ***1323** any definition found in or ascertained by a reading of the patent documents.

Id. at 1584 n. 6.

We also acknowledge that the purpose underlying the *Texas Digital* line of cases--to avoid the danger of reading limitations from the specification into the claim--is sound. Moreover, we recognize that the distinction between using the specification to interpret the meaning of a claim and importing limitations from the specification into the claim can be a difficult one to apply in practice. *See Comark Communications, Inc. v. Harris Corp.*, 156 F.3d 1182, 1186-87 (Fed.Cir.1998) ("there is sometimes a fine line between reading a claim in light of the specification, and reading a limitation into the claim from the specification"). However, the line between construing terms and importing limitations can be discerned with reasonable certainty and predictability if the court's focus remains on understanding how a person of ordinary skill in the art would understand the claim terms. For instance, although the specification often describes very specific embodiments of the invention, we have repeatedly warned against confining the claims to those embodiments. *See, e.g., Nazomi Communications, Inc. v. ARM Holdings, PLC*, 403 F.3d 1364, 1369 (Fed.Cir.2005) (claims may embrace "different subject matter than is illustrated in the specific embodiments in the specification"); *Liebel-Flarsheim*, 358 F.3d at 906-08; *Teleflex*, 299 F.3d at 1327; *SRI Int'l v. Matsushita Elec. Corp. of Am.*, 775 F.2d 1107, 1121 (Fed.Cir.1985). In particular, we have expressly rejected the contention that if a patent describes only a single embodiment, the claims of the patent must be construed as being limited to that embodiment. *Gemstar-TV Guide*, 383 F.3d at 1366. That is not just because section 112 of the Patent Act requires that the claims themselves set forth the limits of the patent grant, but also because persons of ordinary skill in the art rarely would confine their definitions of terms to the exact representations depicted in the embodiments.

To avoid importing limitations from the specification into the claims, it is important to keep in mind that the purposes of the specification are to teach and enable those of skill in the art to make and use the invention and to provide a best mode for doing so.

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See Spectra-Physics, Inc. v. Coherent, Inc., 827 F.2d 1524, 1533 (Fed.Cir.1987). One of the best ways to teach a person of ordinary skill in the art how to make and use the invention is to provide an example of how to practice the invention in a particular case. Much of the time, upon reading the specification in that context, it will become clear whether the patentee is setting out specific examples of the invention to accomplish those goals, or whether the patentee instead intends for the claims and the embodiments in the specification to be strictly coextensive. *See* SciMed Life Sys., 242 F.3d at 1341. The manner in which the patentee uses a term within the specification and claims usually will make the distinction apparent. *See* Snow v. Lake Shore & M.S. Ry. Co., 121 U.S. 617, 630, 7 S.Ct. 1343, 30 L.Ed. 1004 (1887) (it was clear from the specification that there was "nothing in the context to indicate that the patentee contemplated any alternative" embodiment to the one presented).

In the end, there will still remain some cases in which it will be hard to determine whether a person of skill in the art would understand the embodiments to define the outer limits of the claim term or merely to be exemplary in nature. While that task may present difficulties in some cases, we nonetheless believe that attempting to resolve that problem in the context of the ***1324** particular patent is likely to capture the scope of the actual invention more accurately than either strictly limiting the scope of the claims to the embodiments disclosed in the specification or divorcing the claim language from the specification.

[21] In *Vitronics,* this court grappled with the same problem and set forth guidelines for reaching the correct claim construction and not imposing improper limitations on claims. 90 F.3d at 1582. The underlying goal of our decision in *Vitronics* was to increase the likelihood that a court will comprehend how a person of ordinary skill in the art would understand the claim terms. *See id.* at 1584. In that process, we recognized that there is no magic formula or catechism for conducting claim construction. Nor is the court barred from considering any particular sources or required to analyze sources in any specific sequence, as long as those sources are not used to contradict claim meaning that is unambiguous in light of the intrinsic evidence. *See id.* at 1583-84; Intel Corp. v. VIA Techs., Inc., 319 F.3d 1357, 1367 (Fed.Cir.2003). For example, a judge who encounters a claim term while reading a patent might consult a general purpose or specialized dictionary to begin to understand the meaning of the term, before reviewing the remainder of the patent to determine how the patentee has used the term. The sequence of steps used by the judge in consulting various sources is not important; what matters is for the court to attach the appropriate weight to be assigned to those sources in light of the statutes and policies that inform patent law. *Vitronics,* 90 F.3d at 1582. In *Vitronics,* we did not attempt to provide a rigid algorithm for claim construction, but simply attempted to explain why, in general, certain types of evidence are more valuable than others. Today, we adhere to that approach and reaffirm the approach to claim construction outlined in that case, in *Markman,* and in *Innova.* We now turn to the application of those principles to the case at bar.

IV

A

[22] The critical language of claim 1 of the '798 patent-- "further means disposed inside the shell for increasing its load bearing capacity comprising internal steel baffles extending inwardly from the steel shell walls"--imposes three clear requirements with respect to the baffles. First, the baffles must be made of steel. Second, they must be part of the load-bearing means for the wall section. Third, they must be pointed inward from the walls. Both parties, stipulating to a dictionary definition, also conceded that the term "baffles" refers to objects that check, impede, or obstruct the flow of something. The intrinsic evidence confirms that a person of skill in the art would understand that the term "baffles," as used in the '798 patent, would have that generic meaning.

The other claims of the '798 patent specify particular functions to be served by the baffles. For example, dependent claim 2 states that the baffles may be "oriented with the panel sections disposed at angles for deflecting projectiles such as bullets able to penetrate the steel plates." The inclusion of such a specific limitation on the term "baffles" in claim 2 makes it likely that the patentee did not contemplate that the term "baffles" already contained that limitation. *See* Dow Chem. Co. v. United States, 226 F.3d 1334, 1341- 42 (Fed.Cir.2000) (concluding that an independent claim should be given broader scope than a dependent claim to avoid rendering the dependent claim redundant). Independent claim 17 further supports that proposition. It states that baffles are ***1325** placed "projecting inwardly from the outer shell at angles tending to deflect projectiles that penetrate the outer shell." That limitation would be unnecessary if persons of skill in the art understood that the baffles inherently served such a function.

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See TurboCare, 264 F.3d at 1123 (claim terms should not be read to contain a limitation "where another claim restricts the invention in exactly the [same] manner"). Dependent claim 6 provides an additional requirement for the baffles, stating that "the internal baffles of both outer panel sections overlap and interlock at angles providing deflector panels extending from one end of the module to the other." If the baffles recited in claim 1 were inherently placed at specific angles, or interlocked to form an intermediate barrier, claim 6 would be redundant.

The specification further supports the conclusion that persons of ordinary skill in the art would understand the baffles recited in the '798 patent to be load-bearing objects that serve to check, impede, or obstruct flow. At several points, the specification discusses positioning the baffles so as to deflect projectiles. *See* '798 patent, col. 2, II. 13-15; *id.*, col. 5, II. 17-19. The patent states that one advantage of the invention over the prior art is that "[t]here have not been effective ways of dealing with these powerful impact weapons with inexpensive housing." *Id.*, col. 3, II. 28-30. While that statement makes clear the invention envisions baffles that serve that function, it does not imply that in order to qualify as baffles within the meaning of the claims, the internal support structures must serve the projectile-deflecting function in all the embodiments of all the claims. The specification must teach and enable all the claims, and the section of the written description discussing the use of baffles to deflect projectiles serves that purpose for claims 2, 6, 17, and 23, which specifically claim baffles that deflect projectiles. *See* In re Wright, 999 F.2d 1557, 1561 (Fed.Cir.1993).

The specification discusses several other purposes served by the baffles. For example, the baffles are described as providing structural support. The patent states that one way to increase load-bearing capacity is to use "at least in part inwardly directed steel baffles 15, 16." '798 patent, col. 4, II. 14-15. The baffle 16 is described as a "strengthening triangular baffle." *Id.*, col. 4, line 37. Importantly, Figures 4 and 6 do not show the baffles as part of an "intermediate interlocking, but not solid, internal barrier." In those figures, the baffle 16 simply provides structural support for one of the walls, as depicted below:

***1326**

FIG. 4.

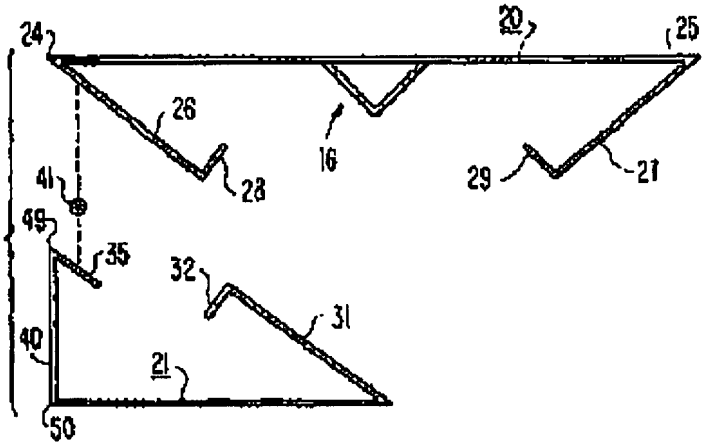
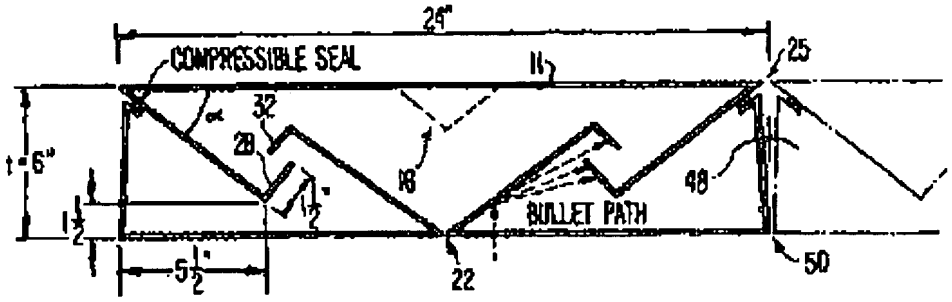


FIG. 6.



Other uses for the baffles are listed in the specification as well. In Figure 7, the overlapping

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flanges "provide for overlapping and interlocking the baffles to produce substantially an intermediate barrier wall between the opposite [wall] faces":

Image 2 not available via Offline Print, but may be viewed on westlaw.com.

'798 patent, col. 5, II. 26-29. Those baffles thus create small compartments that can be filled with either sound and thermal insulation or rock and gravel to stop projectiles. *Id.*, col. 5, II. 29-34. By separating the interwall area into compartments (see, e.g., compartment 55 in Figure 7), the user of the modules can choose different types of material for each compartment, so that the module can be "easily custom tailored for the specific needs of each installation." *Id.*, col. 5, II. 36-37. When material is placed into the wall during installation, the baffles obstruct the flow of material from one compartment to another so that this "custom tailoring" is possible.

[23] The fact that the written description of the '798 patent sets forth multiple ***1327** objectives to be served by the baffles recited in the claims confirms that the term "baffles" should not be read restrictively to require that the baffles in each case serve all of the recited functions. We have held that "[t]he fact that a patent asserts that an invention achieves several objectives does not require that each of the claims be construed as limited to structures that are capable of achieving all of the objectives." *Liebel-Flarsheim*, 358 F.3d at 908; *see also Resonate Inc. v. Alteon Websystems, Inc.*, 338 F.3d 1360, 1367 (Fed.Cir.2003). Although deflecting projectiles is one of the advantages of the baffles of the '798 patent, the patent does not require that the inward extending structures always be capable of performing that function. Accordingly, we conclude that a person of skill in the art would not interpret the disclosure and claims of the '798 patent to mean that a structure extending inward from one of the wall faces is a "baffle" if it is at an acute or obtuse angle, but is not a "baffle" if it is disposed at a right angle.

B

[24] Invoking the principle that "claims should be so construed, if possible, as to sustain their validity," *Rhine v. Casio, Inc.*, 183 F.3d 1342, 1345 (Fed.Cir.1999), AWH argues that the term "baffles" should be given a restrictive meaning because if the term is not construed restrictively, the asserted claims would be invalid.

While we have acknowledged the maxim that claims should be construed to preserve their validity, we have not applied that principle broadly, and we have certainly not endorsed a regime in which validity analysis is a regular component of claim construction. *See Nazomi Communications*, 403 F.3d at 1368-69. Instead, we have limited the maxim to cases in which "the court concludes, after applying all the available tools of claim construction, that the claim is still ambiguous." *Liebel-Flarsheim*, 358 F.3d at 911; *see also Generation II Orthotics Inc. v. Med. Tech. Inc.*, 263 F.3d 1356, 1365 (Fed.Cir.2001) ("[C]laims can only be construed to preserve their validity where the proposed claim construction is 'practicable,' is based on sound claim construction principles, and does not revise or ignore the explicit language of the claims."); *Elekta Instrument S.A. v. O.U.R. Scientific Int'l, Inc.*, 214 F.3d 1302, 1309 (Fed.Cir.2000) ("having concluded that the amended claim is susceptible of only one reasonable construction, we cannot construe the claim differently from its plain meaning in order to preserve its validity"); *E.I. du Pont de Nemours & Co. v. Phillips Petroleum Co.*, 849 F.2d 1430, 1434 (Fed.Cir.1988) (rejecting argument that limitations should be added to claims to preserve the validity of the claims). In such cases, we have looked to whether it is reasonable to infer that the PTO would not have issued an invalid patent, and that the ambiguity in the claim language should therefore be resolved in a manner that would preserve the patent's validity.

That is the rationale that gave rise to the maxim in the first place. In *Klein v. Russell*, 86 U.S. (19 Wall.) 433, 466, 22 L.Ed. 116 (1873), the owner of a reissued patent argued for a narrow construction of the patent, while the accused infringer argued for a broader construction. The Court noted that the law "required that the reissue should be for the same invention as the original patent." *Id.* Because the reissue, which was granted under the predecessor to 35 U.S.C. § 251, would have been improper under the broader construction, the Court "presumed the Commissioner did his duty" and did not issue an invalid patent. For that reason, among others, the Court construed the ***1328** disputed claim language in a manner that "sustain[ed] the patent and the construction claimed by the patentee," since that "can be done consistently with the language which he has employed." *Id.* The applicability of the doctrine in a particular case therefore depends on the strength of the inference that the PTO would have recognized that one claim interpretation would render the claim invalid, and that the PTO would not have issued the patent assuming that to be the proper construction of the term.

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In this case, unlike in *Klein* and other cases in which the doctrine of construing claims to preserve their validity has been invoked, the claim term at issue is not ambiguous. Thus, it can be construed without the need to consider whether one possible construction would render the claim invalid while the other would not. The doctrine of construing claims to preserve their validity, a doctrine of limited utility in any event, therefore has no applicability here.

In sum, we reject AWH's arguments in favor of a restrictive definition of the term "baffles." Because we disagree with the district court's claim construction, we reverse the summary judgment of noninfringement. In light of our decision on claim construction, it is necessary to remand the infringement claims to the district court for further proceedings.

V

With respect to Mr. Phillips's allegation of misappropriation of trade secrets, we agree with the panel's decision upholding the district court's ruling on that issue, in which the district court dismissed the trade secret claim on statute of limitations grounds. *See Phillips*, 363 F.3d at 1214- 1216. Accordingly, based on the panel's disposition of that issue, we affirm the district court's dismissal of the trade secret claim. With respect to AWH's cross-appeal, we also agree with the panel's reasoning and its conclusion that the cross-appeal is improper. *See id.* at 1216. We therefore dismiss the cross-appeal.

VI

In our order granting rehearing en banc, we asked the parties to brief various questions, including the following: "Consistent with the Supreme Court's decision in *Markman v. Westview Instruments*, 517 U.S. 370, 116 S.Ct. 1384, 134 L.Ed.2d 577 (1996), and our en banc decision in *Cybor Corp. v. FAS Technologies, Inc.*, 138 F.3d 1448 (Fed.Cir.1998), is it appropriate for this court to accord any deference to any aspect of trial court claim construction rulings? If so, on what aspects, in what circumstances, and to what extent?" After consideration of the matter, we have decided not to address that issue at this time. We therefore leave undisturbed our prior en banc decision in *Cybor*.

Each party shall bear its own costs for this appeal.

AFFIRMED IN PART, REVERSED IN PART, DISMISSED IN PART, and REMANDED.

LOURIE, Circuit Judge, concurring in part and dissenting in part, with whom PAULINE NEWMAN, Circuit Judge, joins.

I fully join the portion of the court's opinion resolving the relative weights of specification and dictionaries in interpreting patent claims, in favor of the specification. I could elaborate more expansively on that topic, but Judge Bryson's opinion for the majority says it so well, there is little reason for me to repeat its truths. I also agree with the court that claims need not necessarily be limited to specific or preferred embodiments in the specification, although they are limited to what is ***1329** contained in the overall disclosure of the specification.

However, I do dissent from the court's decision to reverse and remand the district court's decision. The original panel decision of this court, which implicitly decided the case based on the priorities that the en banc court has now reaffirmed, interpreted the claims in light of the specification and found that the defendant did not infringe the claims. We affirmed the district court, which had arrived at a similar conclusion. The dissent from the panel decision relied on the "dictionaries first" procedure, which the court now has decided not to follow. Thus, while the claim construction issue had to be decided by the en banc court, I see no reason for the court, having reaffirmed the principle on which the district judge and the panel originally decided the case, to send it back for further review.

The court premises its reverse-and-remand decision on the concept of claim differentiation and the reasoning that the contested term "baffle" need not fulfill all of the functions set out for it in the specification. Reasonable people can differ on those points. However, the court did not take this case en banc because the full court differed with the panel majority on those disputable criteria. It did so to resolve the claim construction issue, which it has now done so well. Having done so, I believe that it should simply affirm the district court's decision on the merits, consistently with that court's rationale and that of the panel that affirmed the district court, which it now adopts.

I will not critique in detail particular statements the majority makes in rationalizing its reversal of the district court's decision, such as "that a person of skill in the art would not interpret the disclosure and claims of the '798 patent to mean that a structure extending inward from one of the wall faces is a 'baffle' if it is at an acute or obtuse angle, but is not a 'baffle' if it is disposed at a right angle," or that "the

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patent does not require that the inward extending structures always be capable of performing that function [deflecting projectiles]" in order to be considered 'baffles'.

I will simply point out that the specification contains no disclosure of baffles at right angles. Moreover, as the majority correctly states, a patent specification is intended to describe one's invention, and it is essential to read a specification in order to interpret the meaning of the claims. This specification makes clear that the "baffles" in this invention are angled. There is no reference to baffles that show them to be other than angled. The abstract refers to "bullet deflecting ... baffles." Only angled baffles can deflect. It then mentions "internal baffles at angles for deflecting bullets." That could not be clearer. The specification then refers several times to baffles, often to figures in the drawings, all of which are to angled baffles. A compelling point is that the only numbered references to baffles (15, 16, 26, 27, 30, and 31) all show angled baffles.

The specification further states that steel panels "form the internal baffles at angles for deflecting bullets." It states that the baffles are "disposed at such angles that bullets which might penetrate the outer steel panels are deflected." It explains that if bullets "were to penetrate the outer steel wall, the baffles are disposed at angles which tend to deflect the bullets." There is no specific reference in this patent to a baffle that is not angled at other than 90.

While, as the majority states, the specification indicates that multiple objectives are achieved by the invention, none of the other objectives is dependent upon whether the baffles are at other than a 90 angle, whereas the constantly stated objective of ***1330** deflection of bullets is dependent upon such an angle.

Finally, even though claim construction is a question of law, reviewable by this court without formal deference, I do believe that we ought to lean toward affirmance of a claim construction in the absence of a strong conviction of error. I do not have such a conviction in this case, after considering the district court's opinion and the patent specification.

For these reasons, while I wholeheartedly join the majority opinion in its discussion and resolution of the "specification v. dictionaries" issue, I would affirm the decision below.

MAYER, Circuit Judge, with whom PAULINE NEWMAN, Circuit Judge, joins, dissenting.

Now more than ever I am convinced of the futility, indeed the absurdity, of this court's persistence in adhering to the falsehood that claim construction is a matter of law devoid of any factual component. Because any attempt to fashion a coherent standard under this regime is pointless, as illustrated by our many failed attempts to do so, I dissent.

This court was created for the purpose of bringing consistency to the patent field. *See* H.R.Rep. No. 312, 97th Cong., 1st Sess. 20-23 (1981). Instead, we have taken this noble mandate, to reinvigorate the patent and introduce predictability to the field, and focused inappropriate power in this court. In our quest to elevate our importance, we have, however, disregarded our role as an appellate court; the resulting mayhem has seriously undermined the legitimacy of the process, if not the integrity of the institution.

In the name of uniformity, *Cybor Corp. v. FAS Technologies, Inc.*, 138 F.3d 1448 (Fed.Cir.1998) (en banc), held that claim construction does not involve subsidiary or underlying questions of fact and that we are, therefore, unbridled by either the expertise or efforts of the district court. [FN1] What we have wrought, instead, is the substitution of a black box, as it so pejoratively has been said of the jury, with the black hole of this court. Out of this void we emit "legal" pronouncements by way of "interpretive necromancy" [FN2]; these rulings resemble reality, if at all, only by chance. Regardless, and with a blind eye to the consequences, we continue to struggle under this irrational and reckless regime, trying every alternative-- dictionaries first, dictionaries second, never dictionaries, etc., etc., etc.

FN1. The Supreme Court did not suggest in affirming *Markman v. Westview Instruments, Inc.*, 52 F.3d 967 (1995) (en banc), that claim construction is a purely legal question. 517 U.S. 370, 116 S.Ct. 1384, 134 L.Ed.2d 577 (1996). It held only that, as a policy matter, the judge, as opposed to the jury, should determine the meaning of a patent claim. *See Cybor*, 138 F.3d at 1464 (Mayer, C.J., dissenting) (explaining that "the [Supreme] Court chose not to accept our formulation of claim construction: as a pure question of law to be decided *de novo* in all cases on appeal").

FN2. *See The Holmes Group, Inc. v. Vornado Air Circulation Sys., Inc.*, 535 U.S.

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826, 833, 122 S.Ct. 1889, 153 L.Ed.2d 13 (2002).

Again today we vainly attempt to establish standards by which this court will interpret claims. But after proposing no fewer than seven questions, receiving more than thirty *amici curiae* briefs, and whipping the bar into a frenzy of expectation, we say nothing new, but merely restate what has become the practice over the last ten years--that we will decide cases according to whatever mode or method results in the outcome we desire, or at least allows us a seemingly plausible way out of the case. I am not surprised by this. ***1331** Indeed, there can be no workable standards by which this court will interpret claims so long as we are blind to the factual component of the task. *See* Cooter & Gell v. Hartmarx Corp., 496 U.S. 384, 405, 110 S.Ct. 2447, 110 L.Ed.2d 359 (1990) ("Fact-bound resolutions cannot be made uniform through appellate review, de novo or otherwise." (quoting *Mars Steel Corp. v. Cont'l Bank N.A.*, 880 F.2d 928, 936 (7th Cir.1989))). [FN3]

FN3. The question asked but not answered by the court which might have allowed it to cure its self-inflicted wound was: "Question 7. Consistent with the Supreme Court's decision in *Markman v. Westview Instruments, Inc.*, 517 U.S. 370, 116 S.Ct. 1384, 134 L.Ed.2d 577 (1996) and our *en banc* decision in *Cybor Corp. v. FAS Technologies, Inc.*, 138 F.3d 1448 (Fed.Cir.1998), is it appropriate for this court to accord any deference to any aspect of trial court claim construction rulings? If so, on what aspects, in what circumstances, and to what extent?"

Federal Rule of Civil Procedure 52(a) states that "[f]indings of fact ... shall not be set aside unless clearly erroneous, and due regard shall be given to the opportunity of the trial court to judge of the credibility of witnesses." According to the Supreme Court, this "[r]ule means what it says"-- that findings of fact, even "those described as 'ultimate facts' because they may determine the outcome of litigation," are to be reviewed deferentially on appeal. [FN4] *Bose Corp. v. Consumers Union of United States*, 466 U.S. 485, 498 & 501, 104 S.Ct. 1949, 80 L.Ed.2d 502 (1984); *see also Anderson v. Bessemer City*, 470 U.S. 564, 575, 105 S.Ct. 1504, 84 L.Ed.2d 518 (1985) ("[R]eview of factual findings under the clearly-erroneous standard--with its deference to the trier of fact--is the rule, not the exception."); *Pullman-Standard v. Swint*, 456 U.S. 273, 287, 102 S.Ct. 1781, 72 L.Ed.2d 66 (1982) ("Rule 52(a) broadly requires that findings of fact not be set aside unless clearly erroneous."); *United States v. United States Gypsum Co.*, 333 U.S. 364, 394, 68 S.Ct. 525, 92 L.Ed. 746 (1948). ***1332** Even those findings of fact based entirely on documentary evidence are entitled to deference. *Anderson*, 470 U.S. at 574, 105 S.Ct. 1504 ("That [Rule 52(a)] goes on to emphasize the special deference to be paid credibility determinations does not alter its clear command: Rule 52(a) 'does not make exceptions or purport to exclude certain categories of factual findings from the obligation of a court of appeals to accept a district court's findings unless clearly erroneous.' " (quoting *Pullman-Standard*, 456 U.S. at 287, 102 S.Ct. 1781)). In short, we are obligated by Rule 52(a) to review the factual findings of the district court that underlie the determination of claim construction for clear error.

FN4. Because some facts are so intertwined with a constitutional standard the Supreme Court has held that *de novo* review is appropriate. For example, whether a defendant has acted with actual malice in a defamation suit is reviewed *de novo* because, among other reasons, the scope of the First Amendment is shaped and applied by reference to such factual determinations. *Bose*, 466 U.S. at 502, 104 S.Ct. 1949 ("[T]he content of the rule is not revealed simply by its literal text, but rather is given meaning through the evolutionary process of common-law adjudication."). Similarly, whether there is reasonable suspicion to conduct an investigatory stop or probable cause to perform a search under the Fourth Amendment are reviewed without deference. *Ornelas v. United States*, 517 U.S. 690, 696, 116 S.Ct. 1657, 134 L.Ed.2d 911 (1996) (holding that the protections afforded by the Fourth Amendment are "fluid concepts that take their substantive content from the particular contexts in which the standards are being assessed"). The reasoning behind these limited exceptions surely does not apply to claim construction. While appearing from the perspective of this court's limited sphere of influence to be dreadfully important, claim construction does not implicate a constitutional value. *Cf. Bose*, 466 U.S. at 502, 104 S.Ct. 1949 ("[T]he constitutional values protected by the rule make it imperative that judges--and in some cases judges of [the Supreme] Court--make sure that it is correctly

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applied."). This is illustrated by the fact that the outcome of a patent case, unlike a defamation or illegal search case, has little impact on how future cases are decided or on how future parties behave. *Cf. id.* at 501 n. 17, 104 S.Ct. 1949 ("Regarding certain largely factual questions in some areas of the law, the stakes--in terms of impact on future cases and future conduct--are too great to entrust them finally to the judgment of the trier of fact."). Even if claim construction did implicate a constitutional value, it, unlike the decisions underlying the First and Fourth Amendments, could readily be reduced, when distinguished from its factual underpinnings, to "a neat set of legal rules." *Ornelas*, 517 U.S. at 695-96, 116 S.Ct. 1657 (quoting *Ill. v. Gates*, 462 U.S. 213, 232, 103 S.Ct. 2317, 76 L.Ed.2d 527 (1983)).

While this court may persist in the delusion that claim construction is a purely legal determination, unaffected by underlying facts, it is plainly not the case. Claim construction is, or should be, made in context: a claim should be interpreted both from the perspective of one of ordinary skill in the art and in view of the state of the art at the time of invention. *See Multiform Desiccants, Inc. v. Medzam, Ltd.*, 133 F.3d 1473, 1477 (Fed.Cir.1998) ("It is the person of ordinary skill in the field of the invention through whose eyes the claims are construed."). These questions, which are critical to the correct interpretation of a claim, are inherently factual. They are hotly contested by the parties, not by resort to case law as one would expect for legal issues, but based on testimony and documentary evidence. [FN5] During so called *Markman* "hearings," which are often longer than jury trials, parties battle over experts offering conflicting evidence regarding who qualifies as one of ordinary skill in the art; the meaning of patent terms to that person; the state of the art at the time of the invention; contradictory dictionary definitions and which would be consulted by the skilled artisan; the scope of specialized terms; the problem a patent was solving; what is related or pertinent art; whether a construction was disallowed during prosecution; how one of skill in the art would understand statements during prosecution; and on and on. In order to reconcile the parties' inconsistent submissions and arrive at a sound interpretation, the district court is required to sift through and weigh volumes of evidence. While this court treats the district court as an intake clerk, whose only role is to collect, shuffle and collate evidence, the reality, as revealed by conventional practice, is far different.

FN5. That most of the cases now appealed to this court are "summary judgments" is irrelevant. We have artificially renamed findings of fact as legal conclusions; the district courts have dutifully conformed to our fictional characterization, but this does not change the inherent nature of the inquiry. Of course, if the parties do not dispute the material facts, summary judgment is appropriate.

Even if the procedures employed by the district court did not show that it is engaging in factfinding, the nature of the questions underlying claim construction illustrate that they are factual and should be reviewed in accordance with Rule 52(a). For each patent, for example, who qualifies as one of ordinary skill in the art will differ, just as the state of the art at the time of invention will differ. These subsidiary determinations are specific, multifarious and not susceptible to generalization; as such their resolution in one case will bear very little, if at all, on the resolution of subsequent cases. *See Ornelas*, 517 U.S. at 703, 116 S.Ct. 1657 ("Law clarification requires generalization, and some issues lend themselves to generalization much more than others."); *Pierce v. Underwood*, 487 U.S. 552, 561-62, 108 S.Ct. 2541, 101 L.Ed.2d 490 (1988) ("Many questions that arise in litigation are not amenable ***1333** to regulation by rule because they involve multifarious, fleeting, special, narrow facts that utterly resist generalization." (quoting Maurice Rosenberg, *Judicial Discretion of the Trial Court, Viewed from Above*, 22 Syracuse L.Rev. 635, 662 (1971))); *Icicle Seafoods, Inc. v. Worthington*, 475 U.S. 709, 714, 106 S.Ct. 1527, 89 L.Ed.2d 739 (1986) (rejecting *de novo* review of factual questions, even when outcome determinative). That the determination of the meaning of a particular term in one patent will not necessarily bear on the interpretation of the same term in a subsequent patent illustrates this point; while the term is the same, the underlying factual context is different. It further proves that these questions (e.g., who qualifies as one of ordinary skill in the art and what was the state of the art at the time of invention, among others) are implicitly being determined in each case; because we refuse to acknowledge either their existence or importance, however, the manner of their resolution is never elucidated. Finally, that claim construction is dependent on underlying factual determinations has been verified by our experience, which shows that reviewing these questions *de novo* has not

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clarified the law, but has instead "distort[ed] the appellate process," causing confusion among the district courts and bar. *See* Cooter, 496 U.S. at 404, 110 S.Ct. 2447 (quoting Pierce, 487 U.S. at 561, 108 S.Ct. 2541); *see also* Koon v. United States, 518 U.S. 81, 99, 116 S.Ct. 2035, 135 L.Ed.2d 392 (1996).

Our purely *de novo* review of claim interpretation also cannot be reconciled with the Supreme Court's instructions regarding obviousness. While ultimately a question of law, obviousness depends on several underlying factual inquiries. Graham v. John Deere Co., 383 U.S. 1, 17, 86 S.Ct. 684, 15 L.Ed.2d 545 (1966); *see also* Dennison Mfg. Co. v. Panduit Corp., 475 U.S. 809, 811, 106 S.Ct. 1578, 89 L.Ed.2d 817 (1986) (holding that Rule 52(a) requires that the district court's subsidiary factual determinations should be reviewed for clear error); *cf.* Graver Tank & Mfg. Co. v. Linde Air Prods. Co., 336 U.S. 271, 275, 69 S.Ct. 535, 93 L.Ed. 672 (1949) (holding that validity, while ultimately a question of law, is founded on factual determinations that are entitled to deference). "Under [section] 103, the scope and content of the prior art are to be determined; differences between the prior art and the claims at issue are to be ascertained; and the level of ordinary skill in the pertinent art resolved." Graham, 383 U.S. at 17, 86 S.Ct. 684.

To a significant degree, each of these factual inquiries is also necessary to claim construction. Before beginning claim construction, "the scope and content of the prior art [should] be determined," *id.*, to establish context. The "differences between the prior art and the claims at issue [should] be ascertained," *id.*, to better define what the inventor holds out as the invention. And, the foundation for both the obviousness and claim construction determinations is "the level of ordinary skill in the pertinent art." *Id.*; *see* Multiform, 133 F.3d at 1477. These underlying factual considerations receive the level of deference due under Rule 52(a) when considering obviousness, but they are scrutinized *de novo* in the claim construction context. As directed by the Supreme Court, however, it is especially important in the patent field, "where so much depends upon familiarity with specific scientific problems and principles not usually contained in the general storehouse of knowledge and experience," to give deference to the district court's findings of fact. Graver Tank & Mfg. Co. v. Linde Air Prods. Co., 339 U.S. 605, 609-10, 70 S.Ct. 854, 94 L.Ed. 1097 (1950).

***1334** While the court flails about in an attempt to solve the claim construction "conundrum," the solution to our plight is straightforward. We simply must follow the example of every other appellate court, which, regarding the vast majority of factual questions, reviews the trial court for clear error. [FN6] This equilibrium did not come about as the result of chance or permissive appellate personalities, but because two centuries of experience has shown that the trial court's factfinding ability is "unchallenged." Salve Regina Coll. v. Russell, 499 U.S. 225, 233, 111 S.Ct. 1217, 113 L.Ed.2d 190 (1991); Inwood, 456 U.S. at 856, 102 S.Ct. 2182 ("Determining the weight and credibility of the evidence is the special province of the trier of fact."). Time has similarly revealed that it is more economical for the district court to find facts. Pierce, 487 U.S. at 560, 108 S.Ct. 2541 ("Moreover, even where the district judge's full knowledge of the factual setting can be acquired by the appellate court, that acquisition will often come at unusual expense, requiring the court to undertake the unaccustomed task of reviewing the entire record").

> FN6. While jurisprudentially sound, the bar also supports this proposition, as evident by the many *amici curiae* briefs urging adherence to Rule 52(a).

Therefore, not only is it more efficient for the trial court to construct the record, the trial court is *better*, that is, more accurate, by way of both position and practice, at finding facts than appellate judges. Anderson, 470 U.S. at 574, 105 S.Ct. 1504 ("The rationale for deference to the original finder of fact is not limited to the superiority of the trial judge's position to make determinations of credibility. The trial judge's major role is the determination of fact, and with experience on fulfilling that role comes expertise."); Zenith Radio Corp. v. Hazeltine Research, Inc., 395 U.S. 100, 123, 89 S.Ct. 1562, 23 L.Ed.2d 129 (1969). Our rejection of this fundamental premise has resulted, not surprisingly, in several serious problems, including increased litigation costs, needless consumption of judicial resources, and uncertainty, as well as diminished respect for the court and less "decisional accuracy." Salve, 499 U.S. at 233, 111 S.Ct. 1217. We should abandon this unsound course. [FN7]

> FN7. There are some scenarios where it is difficult to weed facts from law, *see* Pullman-Standard, 456 U.S. at 288, 102 S.Ct. 1781, but claim construction is not one of them.

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If we persist in deciding the subsidiary factual components of claim construction without deference, there is no reason why litigants should be required to parade their evidence before the district courts or for district courts to waste time and resources evaluating such evidence. It is excessive to require parties, who "have already been forced to concentrate their energies and resources on persuading the trial judge that their account of the facts is the correct one," to "persuade three more judges at the appellate level." *Anderson*, 470 U.S. at 575, 105 S.Ct. 1504. If the proceedings before the district court are merely a "tryout on the road," *id.* (quoting *Wainwright v. Sykes*, 433 U.S. 72, 90, 97 S.Ct. 2497, 53 L.Ed.2d 594 (1977)), as they are under our current regimen, it is wasteful to require such proceedings at all. Instead, all patent cases could be filed in this court; we would determine whether claim construction is necessary, and, if so, the meaning of the claims. Those few cases in which claim construction is not dispositive can be remanded to the district court for trial. In this way, we would at least eliminate the time and expense of the charade currently played out before the district court.

Eloquent words can mask much mischief. The court's opinion today is akin to ***1335** rearranging the deck chairs on the Titanic--the orchestra is playing as if nothing is amiss, but the ship is still heading for Davey Jones' locker.

415 F.3d 1303, 75 U.S.P.Q.2d 1321

Briefs and Other Related Documents **(Back to top)**

- 2004 WL 4991079 (Appellate Brief) EN Bang Brief of Plaintiff-Appellant Edward H. Phillips (Sep. 20, 2004)

- 2004 WL 4991080 (Appellate Brief) Defendants-Cross Appellants' Additional Brief fr Rehearing en Banc (Sep. 20, 2004)

- 2003 WL 25289711 (Appellate Brief) Corrected Reply Brief of Plaintiff-Appellant Edward H. Phillips (Jul. 17, 2003)

- 2003 WL 25289710 (Appellate Brief) Reply Brief of Defendants-Cross Appellants AWH Corporation, Hopeman Brothers, Inc., and Lofton Corporation (Jul. 14, 2003)

- 03-1286 (Docket) (Mar. 12, 2003)

- 03-1269 (Docket) (Feb. 25, 2003)

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United States Court of Appeals,
Federal Circuit.
VITRONICS CORPORATION, Plaintiff-Appellant,
v.
CONCEPTRONIC, INC., Defendant-Appellee.
No. 96-1058.

July 25, 1996.

Patentee brought action against competitor, alleging infringement of its patented method for reflow soldering of surface mounted devices to printed circuit boards. The United States District Court for the District of New Hampshire, Martin F. Loughlin, J., entered judgment in favor of competitor, and patentee appealed. The Court of Appeals, Michel, Circuit Judge, held that: (1) "solder reflow temperature" within meaning of patent meant peak reflow temperature, rather than "liquidus temperature," and (2) trial court erred in relying on expert testimony in interpreting claim.

Reversed and remanded.

West Headnotes

[1] Patents 226.6
291k226.6 Most Cited Cases
Literal patent infringement analysis involves two steps: proper construction of asserted claim and determination as to whether accused method or product infringes asserted claim as properly construed.

[2] Patents 314(5)
291k314(5) Most Cited Cases

[2] Patents 324.5
291k324.5 Most Cited Cases
Patent claim construction is matter of law, which Court of Appeals reviews de novo.

[3] Patents 165(2)
291k165(2) Most Cited Cases

[3] Patents 167(1)
291k167(1) Most Cited Cases

[3] Patents 168(2.1)
291k168(2.1) Most Cited Cases
In interpreting asserted patent claim, court should look first to intrinsic evidence of record, i.e., patent itself, including claims, specification and if in evidence, prosecution history; such intrinsic evidence is most significant source of legally operative meaning of disputed claim.

[4] Patents 165(2)
291k165(2) Most Cited Cases
In interpreting patent claim, Court of Appeals first looks to words of claims themselves, both asserted and nonasserted, to define scope of patented invention.

[5] Patents 162
291k162 Most Cited Cases
Although words in patent claim are generally given their ordinary and customary meaning, patentee may choose to be his or her own lexicographer and use terms in manner other than their ordinary meaning, as long as special definition of term is clearly stated in patent specification or file history.

[6] Patents 167(1.1)
291k167(1.1) Most Cited Cases
In interpreting patent claim, it is always necessary to review specification to determine whether inventor has used any terms in manner inconsistent with their ordinary meaning; specification acts as a dictionary when it expressly defines terms used in claims or when it defines terms by implication.

[7] Patents 167(1)
291k167(1) Most Cited Cases
Patent specification contains written description of invention which must be clear and complete enough to enable those of ordinary skill in the art to make and use it; thus, specification is always highly relevant to claim construction analysis.

[8] Patents 167(1)
291k167(1) Most Cited Cases
Usually, specification is dispositive when discovering patent claims; it is single best guide to meaning of disputed term.

[9] Patents 168(2.1)
291k168(2.1) Most Cited Cases
Court may consider prosecution history of patent, if

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in evidence, in interpreting patent claim.

[10] Patents 168(2.1)
291k168(2.1) Most Cited Cases
"Prosecution history" contains complete record of all proceedings before Patent and Trademark Office (PTO), including any express representations made by patent applicant regarding scope of claims; as such, record before PTO is often of critical significance in determining meaning of claims.

[11] Patents 168(1)
291k168(1) Most Cited Cases
Included within analysis of file history, when interpreting patent claim, may be examination of prior art cited therein.

[12] Patents 159
291k159 Most Cited Cases
In most situations, analysis of intrinsic evidence alone will resolve any ambiguity in disputed patent claim term; in such circumstances, it is improper to rely on extrinsic evidence.

[13] Patents 159
291k159 Most Cited Cases
In those cases where public record unambiguously describes scope of patented invention, reliance on any extrinsic evidence is improper.

[14] Patents 159
291k159 Most Cited Cases
Claims, specification, and file history, rather than extrinsic evidence, constitute public record of patentee's claim, record on which public is entitled to rely.

[15] Patents 227
291k227 Most Cited Cases
Competitors are entitled to review public record, apply established rules of claim construction, ascertain scope of patentee's claimed invention and, thus, design around claimed invention.

[16] Patents 101(2)
291k101(2) Most Cited Cases
"Solder reflow temperature" within meaning of patent for reflow soldering of surface mounted devices to printed circuit boards, meant peak reflow temperature, rather than "liquidus temperature," where preferred embodiment in specification would have fallen outside scope of claim if term was meant to mean "liquidus temperature."

[17] Patents 165(1)
291k165(1) Most Cited Cases
Interpretation of patent so that preferred embodiment falls outside of patent claim is rarely, if ever, correct and would require highly persuasive evidentiary support.

[18] Patents 159
291k159 Most Cited Cases
Even if trial judge permissibly decided to hear all possible evidence before construing patent claim, expert testimony, which was inconsistent with specification and file history, should have been accorded no weight.

[19] Patents 159
291k159 Most Cited Cases
In instances in which intrinsic evidence is insufficient to enable court to determine meaning of certain patent claims, extrinsic evidence may also properly be relied on to understand the technology and to construe claims.

[20] Patents 159
291k159 Most Cited Cases
"Extrinsic evidence" is that evidence which is external to patent and file history, such as expert testimony, inventor testimony, dictionaries, and technical treatises and articles.

[21] Patents 159
291k159 Most Cited Cases
Extrinsic evidence in general, and expert testimony in particular, may be used only to help court come to proper understanding of patent claims; it may not be used to vary or contradict claim language.

[22] Patents 159
291k159 Most Cited Cases
Extrinsic evidence may not contradict import of other parts of patent specifications.

[23] Patents 159
291k159 Most Cited Cases
Where patent documents are unambiguous, expert testimony regarding meaning of claim is entitled to no weight.

[24] Patents 162
291k162 Most Cited Cases
Testimony concerning inventor's objective intent as to claim's scope, when unexpressed in patent documents, cannot guide court to proper

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interpretation of patent when patent documents themselves do so clearly.

[25] Patents 161
291k161 Most Cited Cases
Court in its discretion may admit and rely on prior art proffered by one of the parties, whether or not cited in specification or file history, when interpreting patent; this prior art can often help to demonstrate how disputed claim term is used by those skilled in the art.

[26] Patents 159
291k159 Most Cited Cases
As compared to expert testimony, which often only indicates what particular expert believes patent term means, prior art references may be more indicative of what all those skilled in art generally believe certain term means; reliance on such evidence is unnecessary and improper, however, when disputed terms can be understood from careful reading of public record.

[27] Patents 167(1.1)
291k167(1.1) Most Cited Cases

[27] Patents 168(2.1)
291k168(2.1) Most Cited Cases
Prior art may not be used to vary patent claim terms from how they are defined, even implicitly, in specification or file history.

[28] Patents 162
291k162 Most Cited Cases
Regardless of how those skilled in the art would interpret patent term in other situations, where those of ordinary skill, on reading of patent documents, which conclude that documents preclude term being given meaning propounded by expert witness, court must give it meaning indicated by patentee in patent claim, specification and file history.

[29] Patents 159
291k159 Most Cited Cases
Trial court erred in considering extrinsic evidence when determining meaning of claim term "solder reflow temperature" in patent for method of reflow soldering of surface mounted devices to printed circuit boards, where specification clearly and unambiguously defined term.

[30] Patents 159
291k159 Most Cited Cases
Prior art documents and dictionaries are to be preferred over opinion testimony, whether by attorney or by artisan in field of technology to which patent is directed, when interpreting patent claim.

Patents 328(2)
291k328(2) Most Cited Cases
4,654,502. Cited.
***1578** James J. Foster, Wolf, Greenfield & Sacks, P.C., Boston, Massachusetts, argued, for plaintiff-appellant. With him on the brief were Lawrence M. Green and Brett N. Dorny.

Paul J. Hayes, Weingarten, Schurgin, Gagnebin & Hayes, Boston, Massachusetts, argued, for defendant-appellee. With him on the brief was Dean G. Bostock.

Before MICHEL and LOURIE, Circuit Judges, and FRIEDMAN, Senior Circuit Judge.

MICHEL, Circuit Judge.

Vitronics Corporation ("Vitronics") appeals the September 27, 1995 order of the United States District Court for the District of New Hampshire, Civil Action No. 91-696-L, entering judgment as a matter of law that Vitronics did not prove that Conceptronic, Inc. ("Conceptronic") infringed claim 1 of U.S. Patent No. 4,654,502 ("the '502 patent"). The appeal was submitted for decision after oral argument on May 8, 1996. Because we conclude that the specification of the '502 patent dictates a claim interpretation in accordance with the plaintiff's proposed construction, and that, so construed, the '502 patent may have been infringed, we reverse the trial court's decision and remand for further proceedings.

BACKGROUND

The Patented Invention

Vitronics and Conceptronic both manufacture ovens used in the production of printed ***1579** circuit boards. The ovens are used to solder electrical devices (such as resistors, capacitors and integrated circuits) to the boards. Several methods of soldering devices to boards have been developed; the '502 patent, assigned to Vitronics, is directed to one of those methods.

Specifically, the '502 patent is directed to a method for the reflow soldering of surface mounted devices to a printed circuit board in which the circuit board is moved by a conveyor through a multizone oven. In this process, a solder paste is placed on the circuit board and the devices to be soldered (with attached

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connectors) are placed on the paste. The circuit board is then placed on what is basically a conveyor belt running through an oven and passing through several different heating zones. In the final and hottest zone, the solder paste melts and forms a connection between the device and the circuit board. The boards remain in the last heating zone for only a short duration, allowing the solder to reach a temperature high enough to cause the solder to melt and reflow while maintaining the devices themselves below the solder reflow temperature. Due to this temperature differential, the solder flows up the device connectors to form a solid connection.

Claim 1 of the '502 patent, the only claim at issue in this appeal, reads as follows (with added emphasis on the disputed terms):

> 1. A method for reflow soldering of surface mounted devices to a printed circuit board comprising:
> moving a printed circuit board having solder and devices disposed on a surface thereof through a first zone and in close proximity to a first emitting surface of at least one nonfocused infrared panel emitter, said first emitting surface being at a first panel temperature;
> moving said board through a second zone and in close proximity to a second emitting surface of at least one nonfocused infrared panel emitter, said second emitting surface being at a second panel temperature lower than said first panel temperature; and
> moving said board through a third zone and in close proximity to a third emitting surface of at least one nonfocused infrared panel emitter, said third emitting surface being at a third panel temperature higher than said second panel temperature, said third emitting surface heating said board and said solder to *a solder reflow temperature* for a period of time sufficient to cause said solder to reflow and solder said devices to said board while maintaining the temperature of said devices below *said solder reflow temperature.*

Proceedings Before the District Court

This action was brought on November 26, 1991 by Vitronics against Conceptronic for infringement of both the '502 patent and U.S. Patent No. 4,833,301 ("the '301 patent"). [FN1] At the time the suit was filed, Conceptronic was selling the "Mark series" line of ovens. Conceptronic later discontinued the Mark series and began selling the "HVC series" line of ovens. Prior to trial, the parties stipulated that every limitation of claim 1 of the '502 patent was met by the HVC series of ovens, except the limitation requiring the utilization of "nonfocused infrared panel emitters" and the limitation that the temperature of the devices must be maintained below the "solder reflow temperature." [FN2]

> FN1. A jury returned a verdict of non-infringement of the '301 patent. Vitronics does not appeal that verdict.
>
> FN2. Whether the Conceptronic ovens utilize nonfocused infrared panel emitters is not before this court.

Vitronics, by way of a request for a jury instruction, asked the court to construe the meaning of the "solder reflow temperature" limitation. The specific instruction sought by Vitronics was as follows:

> In considering the question of whether the '502 method patent has been infringed by the Mark and HVC Series ovens, you have to decide whether, in use, those ovens maintain the temperature of the devices below the solder reflow temperature. The phrase "solder reflow temperature" in the '502 patent means the temperature reached by the solder during the period it is reflowing during the final stages of the ***1580** soldering process, sometimes referred to as the "peak solder reflow temperature." It does not mean the "liquidus temperature," the temperature at which the solder first begins to melt. Thus, if the temperature of the devices stays below that of the solder, the '502 method patent is infringed by the Mark and HVC Series ovens.

Thus, Vitronics contended that, as used in the claim, solder reflow temperature means peak reflow temperature, *i.e.*, a temperature approximately 20° C above the liquidus temperature, at which the solder is completely melted and moves freely. Conceptronic, on the other hand, contended that solder reflow temperature means 183° C, *i.e.*, the liquidus temperature of a particular type of solder known as 63/37 (Sn/Pb) solder. [FN3]

> FN3. The specification of the '502 patent describes three exemplary types of solder which can be used in the solder reflow process-- 60/40 (Sn/Pb), 63/37 (Sn/Pb) and 62/36/2 (Sn/Pb/Ag)--each of which, it indicates, has a liquidus temperature of about 190° C and a peak reflow temperature of about 210° to 218° C. At trial, the parties appear to have discussed only 63/37 (Sn/Pb) solder, which has a liquidus temperature of 183° C. However, the claims are not limited to that particular

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> solder or a solder with that particular liquidus temperature.

The district court delayed construing the disputed language until the close of testimony, at which time it ruled in favor of Conceptronic and concluded that the term "solder reflow temperature" as used in claim 1 refers to 183°>>>>>C. Vitronics then conceded that the court was required to grant judgment as a matter of law in favor of Conceptronic, as Vitronics had not presented any evidence of infringement under the court's interpretation of solder reflow temperature. This appeal followed.

Claim Construction Aids Before the District Court

In spite of Vitronics' early request for a jury instruction on the proper claim construction, the district court delayed announcing its claim construction until hearing all the evidence put forth at trial. During trial, and in their briefs to the district court in support of their respective claim constructions, the parties discussed the patent specification, expert testimony, prior testimony and writings of Vitronics and its employees, and technical references. The most pertinent materials are discussed below.

The Patent Specification

Vitronics relied heavily upon the patent itself to support its asserted claim construction. Although Vitronics conceded that the term "solder reflow temperature" may be ambiguous when considered in isolation, it argued that the specification clearly shows that, as used in the claim, solder reflow temperature means peak reflow temperature rather than the liquidus temperature. In particular, Vitronics pointed to that part of the specification that describes a preferred embodiment:

> A preferred embodiment of the invention for reflow soldering of surface mounted devices to printed circuit boards will now be described. The printed circuit boards are typically made of epoxy-glass, such as fire retardant 4(FR-4), or polyamide glass. These boards typically degrade above temperatures of 225° C. The solder may be, for example, 60/40 (Sn/Pb), 63/37 (Sn/Pb), or 62/36/2 (Sn/Pb/Ag), all of which have a liquidus temperature (i.e. begin to melt) of about 190° C. and a peak reflow temperature of about 210°-218° C. Thus, to effect reflow soldering without damaging the board, the solder must be allowed to reach a temperature of at least 210° C., but the board cannot reach a temperature of 225° C.
>
>
>
> The board is then sent into a fifth zone 5 to bring the temperature of the board up to a temperature of approximately 210° C., the devices up to approximately 195° C., and the solder up to approximately 210°>>>>> C. for a period of time of from about 10 to about 20 seconds to cause the solder to flow. Because the devices are cooler than the board, the solder flows up the devices.... The board spends approximately 60 seconds in the fifth zone, but only about 10 to 20 seconds at 210° C. Thus, the board is at the solder reflow temperature for only a short period of time and the ***1581** devices never reach the solder reflow temperature.

Vitronics pointed out that, in the example described as the preferred embodiment, the temperature of the solder is raised to 210° C, the peak reflow temperature, and the temperature of the devices is raised to 195°>>>>> C, 5° above the 190° C liquidus temperature. Thus, as argued by Vitronics, the term "solder reflow temperature" must be construed so that it refers to the peak reflow temperature because the claim requires that the temperature of the devices be maintained below "said solder reflow temperature"; if solder reflow temperature were construed to refer to liquidus temperature, the preferred embodiment would not be covered by the patent claims.

Expert Testimony

Conceptronic relied heavily on the expert testimony of Dr. Rothe. Dr. Rothe testified that the meaning of the term "solder reflow temperature" in claim 1 is synonymous with liquidus temperature. Dr. Rothe further testified that the solder reflow temperature for 63/37 (Sn/Pb) is 183° C. Dr. Rothe likewise testified at trial that several technical articles written by those skilled in the art supported his view that solder reflow temperature refers to liquidus temperature.

The Testimony of Mr. Hall

Conceptronic also relied on the testimony of Mr. Hall, the Chief Engineer at Vitronics. At trial, Mr. Hall confirmed that during his deposition he had testified that the reflow temperature of solder was 183°> C. Mr. Hall also testified that, during his deposition, he had used solder reflow temperature to refer to liquidus temperature. However, at another point in his trial testimony, Hall explained that, while in his earlier deposition testimony he had used solder reflow temperature to refer to liquidus temperature,

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he did not suggest that was how the term was used in the patent. Rather, Hall testified the patent uses the term to refer to the peak reflow temperature.

Paper Written By Former Vitronics Employee

Conceptronic also introduced into evidence a paper written by Phillip Zarrow, a former employee of Vitronics, defining solder reflow temperature in the following manner: "As the temperature of the solder paste on the interconnect passes the solder alloy's melting point and the solder enters a molten state, the assembly enters the reflow region of the process. For 63 Sn/37 Pb, a eutectic solder and the most common SMT alloy, reflow occurs at 183° C." Phillip Zarrow, *Convection/Infrared and Convection Dominant Reflow Soldering of Fine Pitch SMT Devices,* § 10.3.3 (1994). However, that same paper later describes the solder reflow process as taking the temperature of the solder above liquidus: "Most solder manufacturers recommend bringing the interconnection temperature approximately 15 to 25° C above the alloy melting point to achieve full liquidus and assure good solder flow and aid fillet formation." *Id.*

Memorandum of Plaintiff Vitronics Corporation in Opposition to Motion for Summary Judgment of Defendant Conceptronic Corporation and In Support of Plaintiff's Cross-Motion for Summary Judgment of Patent Validity and Infringement

In its brief supporting its proposed construction of claim 1, both at the trial court level and here on appeal, Conceptronic similarly relied on a memorandum written by Vitronics which contains the following language: "Tin/lead solders commonly used by the electronic products industry have a 'liquidus' or 'reflow' temperature in the order of 183° C, or about 361° F." However, this phrase is in the background section of the memorandum and later in the same memorandum, Vitronics discussed the issue of infringement as being whether the temperature of the devices was maintained below "the temperatures of the leads at which the solder is reflowing."

Without indicating which evidence it relied upon, the district court simply ruled that solder reflow temperature meant 183° C.

ANALYSIS

The Use of Intrinsic and Extrinsic Evidence in Claim Construction

[1][2] A literal patent infringement analysis involves two steps: the proper construction ***1582** of the asserted claim and a determination as to whether the accused method or product infringes the asserted claim as properly construed. *Markman v. Westview Instruments, Inc.*, 52 F.3d 967, 976, 34 USPQ2d 1321, 1326 (Fed.Cir.1995) (in banc), *aff'd,* 517 U.S. 370, ----, 116 S.Ct. 1384, 1393, 134 L.Ed.2d 577 (1996); *Hormone Research Found., Inc. v. Genentech, Inc.*, 904 F.2d 1558, 1562, 15 USPQ2d 1039, 1042 (Fed.Cir.1990), *cert. dismissed,* 499 U.S. 955, 111 S.Ct. 1434, 113 L.Ed.2d 485 (1991). The first step, claim construction, is a matter of law, which we review *de novo. Markman,* 52 F.3d at 979, 34 USPQ2d at 1329. Claim construction is the only step in the infringement analysis at issue in this appeal. [FN4]

FN4. No assertion was made that defendant infringed under the doctrine of equivalents.

In determining the proper construction of a claim, the court has numerous sources that it may properly utilize for guidance. These sources have been detailed in our previous opinions, as discussed below, and include both intrinsic evidence (*e.g.,* the patent specification and file history) and extrinsic evidence (*e.g.,* expert testimony).

[3] It is well-settled that, in interpreting an asserted claim, the court should look first to the intrinsic evidence of record, *i.e.,* the patent itself, including the claims, the specification and, if in evidence, the prosecution history. *See Markman,* 52 F.3d at 979, 34 USPQ2d at 1329. Such intrinsic evidence is the most significant source of the legally operative meaning of disputed claim language.

[4][5] First, we look to the words of the claims themselves, both asserted and nonasserted, to define the scope of the patented invention. *See Bell Communications Research, Inc. v. Vitalink Communications Corp.*, 55 F.3d 615, 620, 34 USPQ2d 1816, 1819 (Fed.Cir.1995). Although words in a claim are generally given their ordinary and customary meaning, a patentee may choose to be his own lexicographer and use terms in a manner other than their ordinary meaning, as long as the special definition of the term is clearly stated in the patent specification or file history. *Hoechst Celanese Corp. v. BP Chems. Ltd.*, 78 F.3d 1575, 1578, 38 USPQ2d 1126, 1129 (Fed.Cir.1996) ("A technical term used in a patent document is interpreted as having the meaning that it would be given by persons experienced in the field of the invention, unless it is apparent from the patent and the prosecution history

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that the inventor used the term with a different meaning.") (citations omitted); Hormone, 904 F.2d at 1563, 15 USPQ2d at 1043 ("It is a well-established axiom in patent law that a patentee is free to be his or her own lexicographer and thus may use terms in a manner contrary to or inconsistent with one or more of their ordinary meanings.") (citations omitted).

[6][7][8] Thus, second, it is always necessary to review the specification to determine whether the inventor has used any terms in a manner inconsistent with their ordinary meaning. The specification acts as a dictionary when it expressly defines terms used in the claims or when it defines terms by implication. Markman, 52 F.3d at 979, 34 USPQ2d at 1330. As we have repeatedly stated, "[c]laims must be read in view of the specification, of which they are a part." Id. at 979, 52 F.3d 967, 34 USPQ2d at 1329. The specification contains a written description of the invention which must be clear and complete enough to enable those of ordinary skill in the art to make and use it. Thus, the specification is always highly relevant to the claim construction analysis. Usually, it is dispositive; it is the single best guide to the meaning of a disputed term.

[9][10][11] Third, the court may also consider the prosecution history of the patent, if in evidence. Id. at 980, 52 F.3d 967, 34 USPQ2d at 1330; Graham v. John Deere, 383 U.S. 1, 33, 86 S.Ct. 684, 701-02, 15 L.Ed.2d 545, 148 U.S.P.Q. 459, 473 (1966). This history contains the complete record of all the proceedings before the Patent and Trademark Office, including any express representations made by the applicant regarding the scope of the claims. As such, the record before the Patent and Trademark Office is often of critical significance in determining the meaning of the claims. *See* Markman, 52 F.3d at 980, 34 USPQ2d at 1330; ***1583**Southwall Tech., Inc. v. Cardinal IG Co., 54 F.3d 1570, 1576, 34 USPQ2d 1673, 1676 (Fed.Cir.1995) ("The prosecution history limits the interpretation of claim terms so as to exclude any interpretation that was disclaimed during prosecution.") (citations omitted). Included within an analysis of the file history may be an examination of the prior art cited therein. Autogiro Co. of America v. United States, 181 Ct.Cl. 55, 384 F.2d 391, 399, 155 USPQ 697, 704 (1967) ("In its broader use as source material, the prior art cited in the file wrapper gives clues as to what the claims do not cover.").

[12][13][14][15] In most situations, an analysis of the intrinsic evidence alone will resolve any ambiguity in a disputed claim term. In such circumstances, it is improper to rely on extrinsic evidence. *See, e.g.,* Pall Corp. v. Micron Separations, Inc., 66 F.3d 1211, 1216, 36 USPQ2d 1225, 1228 (Fed.Cir.1995) ("In construing the claims we look to the language of the claims, the specification, and the prosecution history. Extrinsic evidence may also be considered, *if needed* to assist in determining the meaning or scope of technical terms in the claims.") (citations omitted, emphasis added); Hormone, 904 F.2d at 1562, 15 USPQ2d at 1043 ("Claim interpretation involves a review of the specification, the prosecution history, the claims (including unasserted as well as asserted claims), and, *if necessary,* other extrinsic evidence, such as expert testimony.") (citations omitted, emphasis added). In those cases where the public record unambiguously describes the scope of the patented invention, reliance on any extrinsic evidence is improper. The claims, specification, and file history, rather than extrinsic evidence, constitute the public record of the patentee's claim, a record on which the public is entitled to rely. In other words, competitors are entitled to review the public record, apply the established rules of claim construction, ascertain the scope of the patentee's claimed invention and, thus, design around the claimed invention. *See* Markman, 52 F.3d at 978-79, 34 USPQ2d at 1329. Allowing the public record to be altered or changed by extrinsic evidence introduced at trial, such as expert testimony, would make this right meaningless. *See* Southwall, 54 F.3d at 1578, 34 USPQ2d at 1678 ("A patentee may not proffer an interpretation for the purposes of litigation that would alter the indisputable public record consisting of the claims, the specification and the prosecution history, and treat the claims as a 'nose of wax.' " (quoting Senmed, Inc. v. Richard-Allan Med. Indus., Inc., 888 F.2d 815, 819 n. 8, 12 USPQ2d 1508, 1512 n. 8 (Fed.Cir.1989))). The same holds true whether it is the patentee or the alleged infringer who seeks to alter the scope of the claims.

The Proper Construction of the Claim Term "Solder Reflow Temperature"

[16][17] As can be readily seen from those portions of the specification set forth above, the meaning of the disputed term "solder reflow temperature" in claim 1 of the '502 patent is clear from a reading of the claim itself and the patent specification. The "peak reflow temperature" and "liquidus temperature" are clearly defined in the specification as having distinctly different meanings. Specifically, for the solders described in the specification, liquidus temperature is about 190° C and the peak reflow

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temperature is about 210° to 218° C. Moreover, in the preferred embodiment described in the patent, the solder is heated to a temperature of 210° C but the temperature of the devices is maintained at approximately 195° C, *i.e.,* below the peak reflow temperature (210° C) but above the liquidus temperature (190°>> C). Therefore, in order to be consistent with the specification and preferred embodiment described therein, claim 1 must be construed such that the term "solder reflow temperature" means the peak reflow temperature, rather than the liquidus temperature. Indeed, if "solder reflow temperature" were defined to mean liquidus temperature, a preferred (and indeed only) embodiment in the specification would not fall within the scope of the patent claim. Such an interpretation is rarely, if ever, correct and would require highly persuasive evidentiary support, which is wholly absent in this case. *See Modine Mfg. Co. v. United States Int'l Trade Comm'n,* 75 F.3d 1545, 1550, 37 USPQ2d 1609, 1612 (Fed.Cir.1996); *see also Hoechst,* 78 F.3d at 1581, 38 USPQ2d at 1130 ("We ***1584** share the district court's view that it is unlikely that an inventor would define the invention in a way that excluded the preferred embodiment, or that persons of skill in this field would read the specification in such a way.").

The District Court's Reliance on Extrinsic Evidence

[18] Since the claim, read in light of the patent specification, clearly uses the term "solder reflow temperature" to mean the peak reflow temperature, rather than the liquidus temperature, that should have been the end of the trial court's analysis. [FN5] Only if there were still some genuine ambiguity in the claims, after consideration of all available intrinsic evidence, should the trial court have resorted to extrinsic evidence, such as expert testimony, in order to construe claim 1. Moreover, even if the judge permissibly decided to hear all the possible evidence before construing the claim, the expert testimony, which was inconsistent with the specification and file history, should have been accorded no weight. *Southwall,* 54 F.3d at 1578, 34 USPQ2d at 1678; *Markman,* 52 F.3d at 983, 34 USPQ2d at 1333.

> FN5. The file history was apparently not put into evidence.

[19][20][21][22][23][24] Here, the trial judge considered not only the specification, but also expert testimony and other extrinsic evidence, such as the paper written by the former Vitronics employee. No doubt there will be instances in which intrinsic evidence is insufficient to enable the court to determine the meaning of the asserted claims, and in those instances, extrinsic evidence, such as that relied on by the district court, may also properly be relied on to understand the technology and to construe the claims. *See Markman,* 52 F.3d at 979, 34 USPQ2d at 1329. Extrinsic evidence is that evidence which is external to the patent and file history, such as expert testimony, inventor testimony, dictionaries, and technical treatises and articles. [FN6] *Id.* at 980, 34 USPQ2d at 1330. However, as we have recently re-emphasized, extrinsic evidence in general, and expert testimony in particular, may be used only to help the court come to the proper understanding of the claims; it may not be used to vary or contradict the claim language. *Id.* at 981, 52 F.3d 967, 34 USPQ2d at 1331. Nor may it contradict the import of other parts of the specification. Indeed, where the patent documents are unambiguous, expert testimony regarding the meaning of a claim is entitled to no weight. *Southwall,* 54 F.3d at 1578, 34 USPQ2d at 1678. "Any other rule would be unfair to competitors who must be able to rely on the patent documents themselves, without consideration of expert opinion that then does not even exist, in ascertaining the scope of a patentee's right to exclude." *Id.* at 1578, 34 USPQ2d at 1678-79. Nor may the inventor's subjective intent as to claim scope, when unexpressed in the patent documents, have any effect. Such testimony cannot guide the court to a proper interpretation when the patent documents themselves do so clearly.

> FN6. Although technical treatises and dictionaries fall within the category of extrinsic evidence, as they do not form a part of an integrated patent document, they are worthy of special note. Judges are free to consult such resources at any time in order to better understand the underlying technology and may also rely on dictionary definitions when construing claim terms, so long as the dictionary definition does not contradict any definition found in or ascertained by a reading of the patent documents.

[25][26][27] In addition, a court in its discretion may admit and rely on prior art proffered by one of the parties, whether or not cited in the specification or the file history. This prior art can often help to demonstrate how a disputed term is used by those skilled in the art. Such art may make it unnecessary to rely on expert testimony and may save much trial time. As compared to expert testimony, which often

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only indicates what a particular expert believes a term means, prior art references may also be more indicative of what all those skilled in the art generally believe a certain term means. Once again, however, reliance on such evidence is unnecessary, and indeed improper, when the disputed terms can be understood from a careful reading of the public record. *See* Kearns v. Chrysler Corp., 32 F.3d 1541, 1547, 31 USPQ2d 1746, 1750 (Fed.Cir.1994). Nor may it be used to vary claim terms from how ***1585** they are defined, even implicitly, in the specification or file history.

[28][29] Unfortunately, here the trial judge did use the extrinsic evidence to vary or contradict the manifest meaning of the claims. The trial judge was presented with expert testimony and other evidence that some of those skilled in the relevant art, including certain Vitronics employees, sometimes used the term "solder reflow temperature" and "liquidus temperature" interchangeably. He apparently relied on this testimony in reaching his conclusion that, as used in claim 1, solder reflow temperature meant 183°> C. [FN7] However, regardless of how those skilled in the art would interpret a term in other situations, where those of ordinary skill, on a reading of the patent documents, would conclude that the documents preclude the term being given the meaning propounded by the expert witnesses, we must give it the meaning indicated by the patentee in the patent claim, specification and file history. Thus, expert testimony tending to show that those skilled in the art would, in certain circumstances, understand "solder reflow temperature" to mean the solder liquidus temperature is entitled to no weight in light of the clear contrary meaning shown in the specification. *See* Southwall, 54 F.3d at 1578, 34 USPQ2d at 1678 ("Even if Southwall could show that 'sputter-deposited dielectric' has a meaning to one skilled in the art different from the definition in the '745 specification and file history, the definition in the patent documents controls the claim interpretation."). Because the specification clearly and unambiguously defined the disputed term in the claim, reliance on this extrinsic evidence was unnecessary and, hence, legally incorrect.

> FN7. Although the trial judge's reasoning does not appear in the record, he must have relied on the testimony presented by Conceptronic that "solder reflow temperature" and "liquidus temperature" were synonymous and the undisputed testimony that the liquidus temperature of 63/37 (Sn/Pb) solder is 183° C.

[30] Had the district court relied on the expert testimony and other extrinsic evidence solely to help it understand the underlying technology, we could not say the district court was in error. But testimony on the *technology* is far different from other expert testimony, whether it be of an attorney, a technical expert, or the inventor, on the *proper construction* of a disputed claim term, relied on by the district court in this case. The latter kind of testimony may only be relied upon if the patent documents, taken as a whole, are insufficient to enable the court to construe disputed claim terms. Such instances will rarely, if ever, occur. Indeed, this case did not present such an instance. Even in those rare instances, prior art documents and dictionaries, although to a lesser extent, are more objective and reliable guides. Unlike expert testimony, these sources are accessible to the public in advance of litigation. They are to be preferred over opinion testimony, whether by an attorney or artisan in the field of technology to which the patent is directed. Indeed, opinion testimony on claim construction should be treated with the utmost caution, for it is no better than opinion testimony on the meaning of statutory terms. *See* Markman, 52 F.3d at 983, 34 USPQ2d at 1332-33 ("First, the testimony of Markman and his patent attorney on the proper construction of the claims is entitled to no deference.... This testimony about construction, however, amounts to no more than legal opinion--it is precisely the process of construction that the court must undertake.").

Other Issues

Conceptronic further argues that, even if we were to reverse the district court's decision regarding the proper interpretation of the term "solder reflow temperature," the district court's ultimate conclusion of no infringement as a matter of law can still be affirmed on the alternative ground that Vitronics' evidence does not prove infringement because Vitronics failed to test the temperature of all of the various devices on the boards and because certain of the Vitronics tests demonstrated that many of the devices reached temperatures above the peak reflow temperature. Vitronics, of course, disputes these assertions and points to supporting documentation to the effect that the Conceptronic ***1586** ovens do indeed maintain the temperature of the devices below peak reflow temperature. The trial court made no decision on this issue. Moreover, such a determination at this stage would require our weighing substantial but conflicting evidence, an

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impermissible exercise for an appellate court. Accordingly, we must remand.

CONCLUSION

For all the foregoing reasons, the judgment of non-infringement as a matter of law is reversed and the case is remanded for further proceedings consistent with this opinion.

REVERSED AND REMANDED.

COSTS

Costs in favor of Vitronics.

90 F.3d 1576, 65 USLW 2124, 39 U.S.P.Q.2d 1573

END OF DOCUMENT

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H

Briefs and Other Related Documents

United States Court of Appeals,
Federal Circuit.
NOVARTIS PHARMACEUTICALS CORPORATION, Novartis AG, Novartis Pharma AG,
and Novartis International Pharmaceutical Ltd.,
Plaintiffs-Appellants,
v.
ABBOTT LABORATORIES, Defendant-Cross Appellant.
Nos. 03-1367, -1393.

July 8, 2004.
Rehearing and Rehearing En Banc Denied Aug. 31, 2004.

Background: Owner of patent for formulation of pharmaceutical drug cyclosporin sued competitor for infringement. The United States District Court for the District of Delaware, Joseph J. Farnan, Jr., J., 294 F.Supp.2d 557, granted judgment of noninfringement as matter of law, and owner appealed.

Holding: The Court of Appeals, Prost, Circuit Judge, held that patent, whose lipophilic component could include one or more surfactants so long as it also contained at least one non-surfactant lipophilic excipient, was not equivalently infringed by accused product whose only lipophilic component was surfactant.
Affirmed in part and reversed in part.

Bryson, Circuit Judge, dissented and filed opinion.

West Headnotes

[1] **Patents** 226.6
291k226.6 Most Cited Cases

[1] **Patents** 314(5)
291k314(5) Most Cited Cases
In determination of patent infringement, claims must be first construed by court, and then properly construed claims must be compared to accused product by fact finder.

[2] **Patents** 324.5
291k324.5 Most Cited Cases
District court's patent claim construction is reviewed de novo.

[3] **Federal Courts** 776
170Bk776 Most Cited Cases
District court's grant of motion for judgment as matter of law (JMOL) is reviewed de novo. Fed.Rules Civ.Proc.Rule 50, 28 U.S.C.A.

[4] **Courts** 96(7)
106k96(7) Most Cited Cases
Law of regional circuit applies to issue of whether collateral estoppel applies in patent infringement case.

[5] **Judgment** 713(1)
228k713(1) Most Cited Cases

[5] **Judgment** 956(1)
228k956(1) Most Cited Cases
Party seeking to invoke collateral estoppel bears burden of showing: (1) previous determination was necessary to decision; (2) identical issue was previously litigated; (3) issue was actually decided in decision that was final, valid, and on merits; and (4) party being precluded from relitigating issue was adequately represented in previous action.

[6] **Patents** 324.52
291k324.52 Most Cited Cases
Owner of pharmaceutical patents, who failed to appeal from jury's verdict that parent patent was not infringed, was not collaterally estopped from appealing trial court's construction of claim term found in both parent and daughter patents, which had formed basis for court's post-verdict judgment as matter of law (JMOL) that daughter patent was not infringed; it was unclear whether court's construction of term was necessary to jury's noninfringement decision.

[7] **Patents** 162
291k162 Most Cited Cases

[7] **Patents** 165(3)
291k165(3) Most Cited Cases
General presumption that words of patent claim have meaning that person of ordinary skill in relevant art

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would ordinarily attribute to them may be rebutted when: (1) patentee has chosen to be his own lexicographer, or (2) claim term lacks such clarity that there is no means by which scope of claim may be ascertained from language used.

[8] Patents 161
291k161 Most Cited Cases
If disputed patent claim term has no previous meaning to those of ordinary skill in prior art, its meaning must be found elsewhere in patent.

[9] Patents 167(1)
291k167(1) Most Cited Cases
Most often, patent specification is single best guide to meaning of disputed claim term.

[10] Patents 168(2.1)
291k168(2.1) Most Cited Cases
Construing court must examine prosecution history to determine whether patentee relinquished claim coverage by amendment or through argument to overcome or distinguish prior art reference.

[11] Patents 159
291k159 Most Cited Cases
Although extrinsic evidence may be used by construing court to help understand disputed patent claim limitation, it may not be used to vary, contradict, expand, or limit claim language from how it is defined, even by implication, in specification or file history.

[12] Patents 101(2)
291k101(2) Most Cited Cases
"Lipophilic phase component," called for in patent for formulation of
pharmaceutical drug cyclosporin, could include surfactant, but could not be composed entirely of surfactants.

[13] Patents 101(2)
291k101(2) Most Cited Cases
"Lipophilic phase component," called for in patent for formulation of pharmaceutical drug cyclosporin, did not have to be entirely immiscible with hydrophilic phase components in absence of surfactants.

[14] Patents 251
291k251 Most Cited Cases
Patent for formulation of pharmaceutical drug cyclosporin, whose lipophilic component could include one or more surfactants so long as it also contained at least one non-surfactant lipophilic excipient, was not equivalently infringed by accused product whose only lipophilic component was surfactant.

[15] Patents 237
291k237 Most Cited Cases
Doctrine of equivalents may not be applied when accused product's substituted feature places it outside scope of recited claim element.

[16] Patents 324.52
291k324.52 Most Cited Cases
Party who prevails on claim of patent noninfringement has no right to file "conditional" cross-appeal to introduce new argument or challenge claim construction, but may simply assert alternative grounds in record for affirming judgment.

[17] Federal Courts 772
170Bk772 Most Cited Cases
It is only necessary and appropriate for appellee to file cross-appeal when it seeks to enlarge its own rights under judgment or to lessen rights of its adversary under judgment.

Patents 328(2)
291k328(2) Most Cited Cases
5,342,625. Cited.

Patents 328(2)
291k328(2) Most Cited Cases
6,007,840. Not Infringed.
***1329** Robert L. Baechtold, Fitzpatrick, Cella, Harper & Scinto, of New York, NY, argued for plaintiffs-appellants. With him on the brief were Nicholas N. Kallas, Stevan J. Bosses, and Diego Scambia.

Jeffrey I. Weinberger, Munger, Tolles & Olson LLP, of Los Angeles, CA, argued for defendant-cross appellant. With him on the brief were Ted G. Dane and Andrea Weiss Jeffries.

Before BRYSON, GAJARSA, and PROST, Circuit Judges.

Opinion of the Court filed by Circuit Judge PROST. Dissenting opinion filed by Circuit Judge BRYSON.

PROST, Circuit Judge.

Appellants Novartis Pharmaceuticals Corporation, Novartis AG, Novartis Pharma ***1330** AG, and

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Novartis International Pharmaceutical Ltd. (collectively "Novartis") appeal the claim construction and entry by the United States District Court for the District of Delaware of a judgment as a matter of law ("JMOL") for noninfringement of United States Patent No. 6,007,840 ("the '840 patent") in favor of Abbott Laboratories ("Abbott"). *Novartis Pharms. Corp. v. Abbott Labs., Inc.*, 294 F.Supp.2d 557 (D.Del.2003). While we reverse the claim construction of "lipophilic component," we perceive no error in the district court's finding that no reasonable juror could conclude that an equivalent to a pharmaceutically acceptable non-surfactant lipophilic excipient, which is capable of dissolving cyclosporin, is present in Abbott's accused product. Accordingly, we affirm the grant of JMOL for noninfringement.

BACKGROUND

Novartis commenced a lawsuit against Abbott for allegedly infringing United States Patent No. 5,342,625 ("the '625 patent") and the '840 patent. The ' 625 and '840 patents relate to pharmaceutical compositions of the drug cyclosporin, which help prevent organ rejection in transplant patients. Cyclosporin is highly hydrophobic, and as a consequence, has been difficult to administer in a convenient form that provides the desired bioavailability to a patient. '840 patent, col. 3, l. 32, col. 4, ll. 32-40. The two patents asserted by Novartis are directed to compositions that facilitate human absorption of cyclosporin by use of a microemulsion. '625 patent, col. 5, ll. 1-5; '840 patent, col. 4, l. 64-col. 5, l. 1.

After the district court conducted a *Markman* hearing and construed the disputed terms in the '625 and the '840 patents, Novartis narrowed its allegation of infringement in its complaint to claim 1 of the '625 patent [FN1] and claim 81 of the '840 patent [FN2]. Each of the remaining asserted claims calls for a microemulsion pre-concentrate composition that, upon addition of water, forms an oil-in-water ("o/w") microemulsion. The parties disputed, among other things, whether Abbott's product includes a "lipophilic phase component" in the '625 patent or a "lipophilic component" in the '840 patent. In addition, the parties disputed whether "surfactant," as it is used in each of the asserted patents, encompasses ***1331** both hydrophilic and lipophilic surfactants.

> FN1. Claim 1 reads as follows:
> A pharmaceutical composition comprising a cyclosporin as active ingredient,
> 1) a hydrophilic phase component comprising
> 1.1) a pharmaceutically acceptable di- or partial-ether of the formula
> R_1--[O--$(CH_2)_2$]$_x$--OR_2
> wherein R_1 is C_{1-5} alkyl or tetrahydrofurfuryl, R_2 is hydrogen, C_{1-5} » » » » alkyl or tetrahydrofurfuryl, and X is an integer from 1 to 6, or
> 1.2) 1,2-propylene glycol;
> 2) a lipophilic phase component; and
> 3) a surfactant;
> wherein said composition is a microemulsion pre-concentrate, which upon dilution with water to a ratio of 1:1 parts by weight pre-concentrate to water or more of said water, is capable of providing an oil-in-water microemulsion having average particle size of less than about 1,000.

> FN2. Claim 81 reads as follows:
> An oral pharmaceutical composition comprising
> about 5 to about 25% by weight of cyclosporin A, about 0.5 to about 90% by weight of a propylene glycol hydrophilic component, about 0.5 to about 90% by weight of a lipophilic component and about 0.5 to about 90% by weight of a hydrophilic surfactant, all weight percents being based on the total weight of the composition, the relative proportion of said cyclosporin A, hydrophilic component, lipophilic component and hydrophilic surfactant being such that upon dilution of said composition with adequate water, an oil-in-water microemulsion having an average particle size of less than about 1,500 is spontaneously formed.

In its claim construction of the phrase "lipophilic phase component," the district court held that the term requires:

> at least one excipient meeting the following criteria: (1) a pharmaceutically acceptable lipophilic solvent in which cyclosporin is soluble, which is (2) immiscible with both water and the hydrophilic phase component(s) (in the absence of a surfactant), and which (3) lacks the amphiphilic function characteristic of a surfactant (i.e. it *must not be a surfactant*).

Novartis, 294 F.Supp.2d at 561 (emphasis added). The district court construed "surfactant" to encompass both hydrophilic and lipophilic surfactants. While Novartis disputes whether the claim construction of "lipophilic phase component" is

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correct, the parties agree that this term bears the same meaning as the term "lipophilic component," as used in the '840 patent. [FN3]

> FN3. For convenience, we will use the terms "lipophilic phase component" and "lipophilic component" interchangeably.

According to Abbott, its accused product, Gengraf, is a cyclosporin formulation that contains only hydrophilic excipients and surfactants. The accused product contains an ingredient called Span 80, which Abbott contends is a surfactant and not a "lipophilic component." After the parties put on their evidence, the district court permitted Novartis to submit to the jury the question of whether Abbott infringes either of the asserted patents under the doctrine of equivalents. The jury returned a verdict finding that Abbott did not infringe claim 1 of the '625 patent, but did infringe claim 81 of the ' 840 patent. Abbott moved for a judgment as a matter of law ("JMOL") in its favor on claim 81, or in the alternative, a new trial because the district court had erroneously excluded relevant evidence and because the '840 verdict is facially inconsistent with the '625 verdict and against the clear weight of evidence.

The district court granted Abbott's motion for a JMOL. *Id.* at 567. The court noted that under its claim construction, "the 'lipophilic phase component' cannot be a surfactant." *Id.* at 565; *see also id.* at 566 ("Under the Court's claim construction, Novartis is precluded from claiming that surfactants are the equivalent of the 'lipophilic phase component.' "). In construing the claims and concluding that surfactants cannot be a component of the lipophilic phase, the district court relied on a passage from the '625 specification, which states:

> Suitable components for use as lipophilic phase include any pharmaceutically acceptable solvent which is non-miscible with the selected hydrophilic phase, e.g. as defined under (1.1.) or (1.2.). Such solvents will appropriately be devoid or substantially devoid of surfactant function.

'625 patent, col. 8, ll. 58-63. The court effectively read this language as a disavowal of any lipophilic component having surfactant function. In addition, the district court noted that the enumeration of three excipient components in the claim language gave support to viewing each component as separate and distinct. Lastly, the district court observed that the patentee had acknowledged during the prosecution of the application that led to the ' 840 patent that distinctions exist between surfactant and lipophilic compounds that differentiate the chemical entity of each class of compound. According to the district court, "[b]ecause surfactants are expressly excluded from coverage, a surfactant acting as the 'lipophilic phase component'***1332** is inconsistent with the language of the claim, as construed by the Court." *Novartis*, 294 F.Supp.2d at 567. Because Span 80 is a surfactant, the district court reasoned that Novartis could not, as a matter of law under the specific exclusion principle, establish that the substance is an equivalent to the "lipophilic component." *Id.* Accordingly, the district court concluded that Novartis could not demonstrate that Abbott infringes under the doctrine of equivalents. *Id.*

STANDARD OF REVIEW

[1][2][3] In a determination of infringement, the claims must be first construed by the court, and then the properly construed claims must be compared to the accused product by the fact finder. *Cybor Corp. v. FAS Techs. Inc.*, 138 F.3d 1448, 1454 (Fed.Cir.1998) (en banc). If challenged, we review claim construction de novo. *Golight, Inc. v. Wal-Mart Stores, Inc.*, 355 F.3d 1327, 1330 (Fed.Cir.2004); *Markman v. Westview Instruments, Inc.*, 52 F.3d 967, 970 (Fed.Cir.1995) (en banc), *aff'd*, 517 U.S. 370, 116 S.Ct. 1384, 134 L.Ed.2d 577 (1996). We likewise review the grant of a JMOL for noninfringement de novo, reapplying the JMOL standard used by the district court. *Ericsson, Inc. v. Harris Corp.*, 352 F.3d 1369, 1373 (Fed.Cir.2003); *Markman*, 52 F.3d at 975. "To prevail on a renewed motion for JMOL following a jury trial, a party 'must show that the jury's findings, presumed or express, are not supported by substantial evidence or, if they were, that the legal conclusion(s) implied [by] the jury's verdict cannot in law be supported by those findings.' " *Pannu v. Iolab Corp.*, 155 F.3d 1344, 1348 (Fed.Cir.1998) (citing *Perkin-Elmer Corp. v. Computervision Corp.*, 732 F.2d 888, 893 (Fed.Cir.1984)); Fed.R.Civ.P. 50(b). If the former situation applies, "we must view the evidence in the light most favorable to the non-moving party, and determine whether the record contains the 'minimum quantum of evidence from which a jury might reasonably afford relief.' " *Glenn Distribs. Corp. v. Carlisle Plastics, Inc.*, 297 F.3d 294, 299 (3d Cir.2002) (quoting *Parkway Garage, Inc. v. City of Philadelphia*, 5 F.3d 685, 691 (3d Cir.1993)).

DISCUSSION

I. Collateral Estoppel

As a threshold issue, Abbott asserts that Novartis is barred in this appeal from challenging the jury verdict on the '625 patent or the JMOL on the ' 840 patent. Because the parties agree that the terms

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"lipophilic component" and "surfactant" are common to the '625 and '840 patents, Abbott argues that Novartis' failure to challenge the construction of these terms in connection with the '625 patent estops Novartis from challenging their meaning in the related '840 patent. According to Abbott, "any legal determinations or factual findings necessary to the jury's verdict as to claim 1 of the '625 patent, the parent of the '840 patent, must be accorded preclusive effect." (Citing *Mycogen Plant Science, Inc. v. Monsanto Co.*, 252 F.3d 1306, 1311 (Fed.Cir.2001)).

Novartis claims that it preserved its right to challenge the jury verdict on the '625 patent, and that collateral estoppel does not apply to preclude its challenge to the JMOL on the '840 patent. As evidence, Novartis notes that when discussing the construction of the term "surfactant," it pointed out that the correct construction of this term is relevant only to the '625 patent. Novartis further observes that it requested that "if this Court reverses the district court's claim construction" and "if this Court does not reinstate the jury [infringement] verdict," then "it is requested that ... the case [be] remanded for further proceedings consistent with the ***1333** proper construction of the claim terms." According to Novartis, the "further proceedings" necessarily include proceedings on claim 1 of the '625 patent as well as claim 81 of the '840 patent. Novartis moreover notes that Abbott admitted that the Notice of Appeal may be broad enough to encompass the appeal of the jury verdict on the '625 patent. In sum, Novartis asserts that (1) its conditional request of this court to construe a term for the '625 patent, (2) its request to remand "for further proceedings," and (3) Abbott's concession that the Notice of Appeal may be sufficiently broad to encompass an appeal of the '625 patent verdict, suffice to show that it preserved its right to challenge the claim construction, the noninfringement verdict on the ' 625 patent, and the JMOL that followed.

[4][5] We apply the law of the regional circuit to the issue of collateral estoppel in circumstances such as those presented here. *RF Del., Inc. v. Pac. Keystone Techs., Inc.*, 326 F.3d 1255, 1261 (Fed.Cir.2003). The party seeking to invoke collateral estoppel bears the burden, *Dici v. Pennsylvania*, 91 F.3d 542, 548 (3d Cir.1996), of showing the following four elements are present: "(1) the previous determination was necessary to the decision; (2) the identical issue was previously litigated; (3) the issue was actually decided in a decision that was final, valid, and on the merits; and (4) the party being precluded from relitigating the issue was adequately represented in the previous action." *Hawksbill Sea Turtle v. FEMA*, 126 F.3d 461, 475 (3d Cir.1997) (citing *Raytech Corp. v. White*, 54 F.3d 187, 190 (3d Cir.1995)).

Starting with Novartis' contention that its appeal of the jury verdict on the '625 patent was preserved, we note that Novartis, from the beginning, has maintained that "this is an *appeal from entry of JMOL* overturning a jury verdict finding Abbott guilty of infringement under the doctrine of equivalents." (Emphasis added). According to Novartis, "[t]he element at the core of this appeal (in that it formed the basis for the district court's *grant of JMOL*) is the 'lipophilic component' element." (Emphasis added). As a consequence of the district court's allegedly erroneous claim construction, Novartis urged this court to "overturn the *JMOL ruling* " and reinstate the jury's verdict. (Emphasis added). Novartis urged that in the alternative, if this court decides against reinstating the verdict, but rather "orders a retrial, [this court] should construe the term 'surfactant' *to provide guidance* " to the district court. (Emphasis added). The purpose of examining the term "surfactant," therefore, is not to attack the '625 patent jury verdict, as Novartis belatedly now contends in response to Abbott's collateral estoppel argument, but rather to provide guidance to the district court if we order a retrial of the '840 patent case. In support of its proposed alternative, Novartis cited *Altiris, Inc. v. Symantec Corp.*, 318 F.3d 1363, 1366 (Fed.Cir.2003), as a parallel situation. There, this court consented to construe terms that *did not* form a part of the appealed summary judgment because they "may be relevant to the remand determination of infringement" and because they were found to be construed erroneously. *Id.* We find no language in Novartis' opening brief stating that this is also an appeal from the jury verdict on the '625 patent or arguments explaining why the allegedly erroneous claim construction compels us to conclude that the jury's noninfringement verdict on the '625 patent mandates a reversal. It is our understanding from Novartis' statement of its position that "this is an appeal from entry of JMOL" and that this court need not broach the meaning of "surfactant," which itself does not form a part of the appealed ***1334** JMOL, unless this court remands the case for a retrial. For reasons discussed below, we affirm the JMOL, and thus we find remand unwarranted. Based on our affirmance, our review of the term "surfactant," as it pertains to the '625 patent, is uncalled for.

[6] As to collateral estoppel, we find it inapplicable to the JMOL on the '840 patent. Here, the jury

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determined that Novartis failed to prove by a preponderance of evidence that Abbott infringed the '625 patent under the doctrine of equivalents. The verdict form on which the jury indicated its decision did not require the jury to specify the one or more limitations in claim 1 that it found Abbott's product did not contain. Further, there is no record evidence explaining the jury's rationale for its verdict. Assuming that the district court's claim construction of "lipophilic component" was the dispositive element that proved necessary to the jury's noninfringement verdict, as Abbott urges, it would appear that the jury's infringement verdict on the '840 patent is quite possibly contradictory. One explanation may be as Novartis suggests. According to Novartis, the jury may have "include[d] all of the SPAN® 80 and CREMOPHOR® EL ingredients within the 'surfactant' component of the ['625] claim, thereby leaving no ingredient to serve as the lipophilic component." Because claim 81 called more specifically for a hydrophilic surfactant, the jury could have concluded that Cremophor EL is the only surfactant that meets the hydrophilic surfactant element, leaving the jury free to conclude that the unaccounted for Span 80 was, based on testimony from Novartis' expert, not acting as a surfactant but rather as a lipophilic component in the claimed composition. In any event, we cannot conclude from the limited information available that the district court's construction of "lipophilic phase component," proved necessary to the jury's noninfringement decision on the '625 patent. Accordingly, Abbott has not met its burden to show that the claim construction of that term by the district court was necessary to the jury's noninfringement decision. Collateral estoppel therefore should not apply to preclude Novartis from challenging the claim construction and the JMOL in regards to the '840 patent.

II. Claim Construction

[7] In construing claims, our analysis must begin and remain centered on the claim language itself. *Interactive Gift Express, Inc. v. Compuserve, Inc.* 256 F.3d 1323, 1331 (Fed.Cir.2001). We generally presume that the words of a patent claim have the meaning that a person of ordinary skill in the relevant art would ordinarily attribute to them. *Tex. Digital Sys., Inc. v. Telegenix, Inc.*, 308 F.3d 1193, 1202 (Fed.Cir.2002); *Johnson Worldwide Assocs., Inc. v. Zebco Corp.*, 175 F.3d 985, 989 (Fed.Cir.1999). The presumption may be rebutted when: (1) the patentee has chosen to be his own lexicographer, or (2) a claim term lacks such clarity that there is "no means by which the scope of the claim may be ascertained from the language used." *Johnson Worldwide*, 175 F.3d at 990.

[8][9][10][11] If the disputed claim term "is a term with no previous meaning to those of ordinary skill in the prior art[,][i]ts meaning, then, must be found [elsewhere] in the patent." *J.T. Eaton & Co. v. Atl. Paste & Glue Co.*, 106 F.3d 1563, 1568 (Fed.Cir.1997). Most often, the specification "is the single best guide to the meaning of a disputed term." *Vitronics Corp. v. Conceptronic, Inc.*, 90 F.3d 1576, 1582 (Fed.Cir.1996). Even when guidance is not provided in explicit definitional format, "the specification may define claim terms 'by implication' such that the meaning may be 'found in or ascertained by a reading of the patent documents.' " ***1335***Bell Atl. Network Servs., Inc. v. Covad Communications Group, Inc.*, 262 F.3d 1258, 1268 (Fed.Cir.2001) (quoting *Vitronics*, 90 F.3d at 1582, 1584 n. 6). In addition to the specification, we must examine the prosecution history to determine whether the patentee relinquished claim coverage by amendment or through argument to overcome or distinguish a reference. *Id.* Although extrinsic evidence may be used by the court to help understand the disputed limitation, it may not be used to vary, contradict, expand, or limit the claim language from how it is defined, even by implication, in the specification or file history. *Vitronics*, 90 F.3d at 1584-85.

Novartis claims that the district court erred by not giving the term "lipophilic component" the broadest meaning possible. More specifically, Novartis asserts the district court unnecessarily added limitations concerning whether the "lipophilic component" may include surfactants and whether the constituents of the lipophilic component must be immiscible.

A.

[12] Novartis begins its analysis by observing that "lipophilic" has a well-defined meaning to those skilled in the art. As defined by Novartis' expert, lipophilic means "fat- or oil-loving, or water hating." Novartis then notes that the specification teaches that:

> The relative proportions will also vary, depending on the particular function of ingredients in the composition, for example, in the case of a surfactant component of a "microemulsion pre-concentrate", on whether this is employed as a surfactant only or both a surfactant and a co-solvent.

'840 patent, col. 16, ll. 36-41. Further, the specification teaches that:

> When the surfactant comprises an effective solvent for the cyclosporin active ingredient, as in the case

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> e.g. of surfactants or mixtures of surfactants under (3.1.1.) to (3.2.7.) above, it may be incorporated into compositions as defined under (A), not only as surfactant, but in excess as an additional carrier or co-solvent phase, i.e. *as part of the* hydrophilic or *lipophilic phase.*

Id., col. 12, ll. 44-49. Novartis claims that these teachings are inconsistent with the court's construction that a surfactant can form no part of the "lipophilic component." We agree with Novartis.

Claim 81 of the '840 patent recites a pharmaceutical composition that forms a microemulsion upon dilution by water. The composition comprises, in addition to the active ingredient, three other components. Namely, it contains (1) a hydrophilic component, (2) a "lipophilic component," and (3) a hydrophilic surfactant. Neither party cites any persuasive record evidence that the term "lipophilic component" (or "lipophilic phase component") had a well-defined meaning to those of ordinary skill in the art. Accordingly, the claim term requires that we turn to the intrinsic evidence, and if necessary, the extrinsic evidence, to elucidate the meaning that one skilled in the art would give the term. *Johnson Worldwide*, 175 F.3d at 990.

The passages of the specification quoted above indicate that a surfactant or a mixture of surfactants, provided it is an "effective solvent for the cyclosporin," may form, contrary to the district court's construction, a *part* of the lipophilic phase that dissolves the cyclosporin. Furthermore, the ' 840 patent discloses that if a surfactant forms a part of the lipophilic phase, it does so as a *co-solvent.* Col. 12, ll. 43-49, col. 16, ll. 36-41. A co-solvent is a "solvent that in conjunction with *another solvent* can dissolve a solute." *Webster's Third New International Dictionary* ("*Webster's* ") 514 (1993) (emphasis added). Novartis' ***1336** expert opines that the specification's disclosure indicates that a surfactant "could function *alone* " as the solvent in the lipophilic phase. (Emphasis added). By definition, however, a co-solvent operates with "another solvent" to dissolve a solute, such as cyclosporin. Novartis' expert testimony therefore directly contradicts the express teaching of the specification, and may not be used to give us appropriate guidance to reach the correct claim construction.

At oral argument, Novartis attempted to make a similar argument, asserting that the description of surfactants as making up only a "part" of the lipophilic phase, *see* '840 patent, col. 12, ll. 48-49, indicates that surfactants could constitute the whole lipophilic phase. "Part," however, means "something less than a whole." *Webster's* at 1645. Because Novartis supplies insufficient record evidence to validate its unconventional interpretations of "co-solvent" and "part," as those words are used in the specification, Novartis' reading of the specification must be rejected.

In an attempt to further support its view that surfactants may constitute the entire lipophilic phase, Novartis notes that Example 4.1 in the '840 patent describes a "regular emulsion pre-concentrate" in which only a surfactant is used to dissolve the cyclosporin. The composition defined by claim 81 is not a regular emulsion pre-concentrate, however, but rather a microemulsion pre-concentrate, and therefore the example does nothing to advance our understanding of what composes the "lipophilic component" in the claimed composition. [FN4]

> FN4. For the same reason, we must reject the assertion by Novartis' expert that the written description in the '840 patent at column 14, lines 39-44 provides further evidence that surfactants may form a part of the lipophilic component. The compositions as defined under (C) of the specification provide emulsion pre-concentrates and are directed to compositions "other than those as defined under (A) and (B)," (both of which are microemulsion pre-concentrates) to form "for example, solutions, suspensions, dispersion[,] regular emulsions and the like." '840 patent, col. 14, ll. 45-54.

Because the specification teaches that "*mixtures* of surfactants," if used to dissolve the cyclosporin, form only a "part" of the lipophilic phase, '840 patent, col. 12, ll. 43-49 (emphasis added), the other solvent forming the rest of the lipophilic phase must not be another surfactant. Rather, according to the specification, the remainder of the solvent constituting the lipophilic phase is a pharmaceutically acceptable non-surfactant lipophilic excipient that is capable of dissolving cyclosporin, such as those described under section (2) of the specification. *Id.*, col. 8, ll. 66-67; col. 9, ll. 5-46; col. 12, ll. 43-49. Accordingly, while we agree with Novartis that surfactants may form a part of the lipophilic component, the intrinsic record shows that this component cannot be composed entirely of surfactants, as urged by Novartis. Although Abbott initially argued in its opposition brief that the '840 patent "requires the lipophilic (phase) component to be comprised of an excipient other than a surfactant,"

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Abbott later admitted in its cross-appeal brief and at oral argument that "a surfactant [may] contribute[] to the lipophilic phase so long as the lipophilic phase *also* contained at least one non-surfactant excipient." In view of the intrinsic evidence, the claimed "lipophilic component" must contain, at minimum, a pharmaceutically acceptable lipophilic substance capable of dissolving cyclosporin, and that is a non-surfactant excipient.

B.

[13] Novartis also challenges the district court's inclusion of the requirement that the lipophilic component must be immiscible ***1337** with the hydrophilic phase in the absence of surfactants. The specification states that "suitable *components* " for use in the lipophilic phase may include any pharmaceutically acceptable solvent that is non-miscible with the selected hydrophilic phase. '840 patent, col. 9, ll. 1-3 (emphasis added). The specification, contrary to the district court's reading, does not teach that the entire lipophilic phase must be immiscible with the hydrophilic phase, but rather only components, *e.g.,* parts, of the phase, in one embodiment, should be immiscible. We find nothing within the intrinsic record that compels reading the immiscibility limitation into the "lipophilic component" term.

III. Jury Verdict

Novartis asserts that even if we uphold the district court's construction of "lipophilic component," the jury's verdict on the '840 patent should be allowed to stand because there was substantial evidence for the jury to conclude that Abbott's cyclosporin composition infringed under the doctrine of equivalents. In granting JMOL, Novartis argues that the district court compounded the error of its claim construction by erroneously applying the specific exclusion principle and the all-elements rule. Moreover, Novartis suggests that even if we allow the claim construction to stand, the latter errors mandate a reversal of the JMOL.

In light of its exclusion of surfactants from the lipophilic component, the district court found that the specific exclusion principle precluded it, as a matter of law, from treating the surfactant as an element forming the lipophilic component. *Novartis,* 294 F.Supp.2d at 567. Left with no identifiable compound in the accused product corresponding to the lipophilic component, the district court held, under the all-elements rule, that no reasonable fact finder could conclude that Abbott infringes under the doctrine of equivalents. *Id.*

[14] As reviewed above in connection with claim construction, the specification informs us that the lipophilic component encompasses compositions that may include one or more surfactants, so long as the lipophilic component *also* contains at least one non-surfactant lipophilic excipient, which is capable of dissolving cyclosporin. Novartis argued that Span 80, which is a surfactant and an ingredient of the accused product, meets the lipophilic component limitation under the doctrine of equivalents. Novartis cites the testimony of its expert who opined at trial that Span 80 "*does not* exhibit the amphiphilic *function* of a surfactant" nor does it "*function* as a surfactant ... but, rather, that it *functions* as the lipophilic component." (Emphases added). The fact that Span 80 may "function" as a co-solvent to cyclosporin does not answer, however, whether a suitable non-surfactant excipient is also present in the accused composition, thereby bringing the alleged co-solvent mixture within the scope of the term "lipophilic component," either literally or under the doctrine of equivalents.

In light of the specification's implicit teaching that surfactants do not compose the entire portion of the lipophilic component, Novartis is foreclosed from arguing that Span 80, which the specification expressly acknowledges is a surfactant, '840 patent, col. 9, ll. 49-50; col. 11, ll. 58-62, is an equivalent to a pharmaceutically acceptable *non-surfactant* lipophilic excipient, as required by the lipophilic phase under our claim construction. *See SciMed Life Sys., Inc. v. Advanced Cardiovascular,* 242 F.3d 1337, 1347 (Fed.Cir.2001) ("[I]f a patent states that the claimed device must be 'non-metallic,' the patentee cannot assert the patent against a metallic device on the ground that a metallic device is equivalent to a non-metallic device."); *Durel Corp. v. Osram Sylvania Inc.,* 256 F.3d 1298, 1305 (Fed.Cir.2001) ("A finding of equivalence ***1338** [with Sylvania's AlO(OH) and $Al(OH)_3$ coatings] would vitiate the limitation 'oxide coating,' which we have concluded is defined to primarily consist of a binary compound."); *see also Athletic Alternatives, Inc. v. Prince Mfg. Inc.,* 73 F.3d 1573, 1582 (Fed.Cir.1996) ("As a corollary to the 'all limitations' rule ..., we have held that 'the concept of equivalency cannot embrace a structure that is specifically excluded from the scope of the claims.' " (citation omitted)).

Novartis claims that the district court's holding that Span 80 may not, as a matter of law, be regarded as an equivalent to a non-surfactant excipient under the

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asserted patents is contrary to the Supreme Court's decision in *Graver Tank & Manufacturing Co. v. Linde Air Products Co.*, 339 U.S. 605, 70 S.Ct. 854, 94 L.Ed. 1097 (1950), and this court's decision in *Wright Medical Technology, Inc. v. Osteonics Corp.*, 122 F.3d 1440 (Fed.Cir.1997). We disagree. In *Graver Tank,* the patentees asserted four claims directed to welding flux made of alkaline earth metal silicates. 339 U.S. at 607, 70 S.Ct. 854. Except for the type of metal silicate present, the accused product was alike in all respects to the claimed welding flux. *Id.* at 610, 70 S.Ct. 854. The parties disputed whether silicates made of manganese metal in the accused product could be treated as an equivalent to silicates made of alkaline earth metal. *Id.* The Court found that the difference between the metal silicates was insubstantial, and they therefore could be considered equivalents. *Id.* Notably, there was no dispute that the metal silicate element was present in the accused product. Rather, the dispute focused on a limitation of the metal silicate element.

Similarly, in *Wright Medical,* there was no dispute that the accused product employed intramedullary rod elements, just as in the claimed invention. 122 F.3d at 1445. The asserted independent claim defined a femoral surface shaping guide comprising "an intramedullary rod portion adapted to *closely fit in* and *extend through* the narrowest portion of the human femur." *Id.* at 1442 (emphases added). The district court construed "closely fit" to mean "a tight or snug fit between the rod and the isthmus of the femur" and "extend through" to require that the "rod pass through the entire length of isthmus." *Id.* at 1444. Under this claim construction, the parties agreed that the intramedullary rods of the accused product did not fit tightly against or extend through the isthmus of the femur, and thus the product did not literally infringe the asserted patent. *Id.* The parties disputed, however, whether the rods in the accused product were insubstantially different from that claimed, and thus, whether the accused product infringed under the doctrine of equivalents. *Id.* at 1445. The district court granted summary judgment to the accused infringer, rejecting the patentee's theory for infringement by equivalence because the theory "attempt[ed] to ignore claim limitations on which the public is entitled to rely." *Id.* We found that summary judgment for the accused infringement was improper, and held that the fact finder must be given the opportunity to determine whether the intramedullary rod in the accused product is equivalent to that used in the claimed invention. *Id.* at 1446.

In both *Graver Tank* and *Wright Medical,* the fact that certain claimed limitations in the element at issue were missing in the accused product did not change the fact that the element, albeit different from that expressly claimed, was indeed present in the accused product. In *Graver Tank,* for instance, although the accused product used manganese metal instead of an alkaline earth metal, that substitution did not transform the disputed element, i.e ., metal silicate, into something that was not a metal silicate. Similarly, in *Wright Medical,* ***1339** although the intramedullary rod in the accused product did not tightly fit and extend through the isthmus of the femur, as required by the literal claim language, the absence of those claimed limitations did not vitiate the fact that the accused product possessed an "intramedullary rod."

[15] When the substitution of one feature, however, for another into an element of the accused product places it outside the scope of the recited claim element, the doctrine of equivalents may not be applied. *Sage Prods., Inc. v. Devon Indus., Inc.*, 126 F.3d 1420, 1423 (Fed.Cir.1997); *see, e.g., Durel Corp.*, 256 F.3d at 1305 (finding that the addition of hydroxyl groups to the alleged oxide coating in the accused product made it no longer an "oxide coating" as properly construed under the asserted patent, and thus placing the accused product outside the reach of the doctrine of equivalents). Permitting such an element in the accused product to come within the bounds of the claimed element would impermissibly extend the scope of the claim language beyond what the patentee actually claimed.

Here, the "lipophilic component" element requires at least a pharmaceutically acceptable non-surfactant lipophilic excipient capable of dissolving cyclosporin. Under Novartis' theory of infringement by equivalence, Span 80, admittedly a surfactant according to the '840 patent, *see* col. 9, ll. 47-50, col. 11, ll. 58-59, is an equivalent to the *non-surfactant* excipient required by the claimed "lipophilic component." Including a chemical composition made entirely of surfactants within the scope of "lipophilic component" would be inconsistent with the claim construction of that term. We must reject Novartis' theory of infringement by equivalence. Because Novartis failed to adduce evidence from which a reasonable juror could conclude, consistent with this court's claim construction, that there is a "lipophilic component" in Abbott's accused product, we find no basis to reverse the JMOL for noninfringement of the '840 patent.

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IV. Cross-Appeal

In Abbott's conditional cross-appeal, it claims that in the event we reverse the JMOL, we should not reinstate the jury verdict. Rather, Abbott urges this court to grant it a new trial on the '840 patent, because the jury verdict of infringement of the '840 patent is irreconcilably inconsistent with the jury verdict of noninfringement as to the '625 patent. In addition to the inconsistency, Abbott contends that it is entitled to a new trial on the ' 840 patent because the jury's verdict is contrary to the clear weight of the evidence and because the district court erroneously excluded evidence that Abbott holds its own patents for its accused product.

[16][17] The district court granted Abbott's motion for JMOL for noninfringement and denied Abbott's alternative motion for a new trial as moot. *Novartis*, 294 F.Supp.2d at 560. "[A] party who prevails on noninfringement has no right to file a 'conditional' cross-appeal to introduce new argument or challenge a claim construction, but may simply assert alternative grounds in the record for affirming the judgment." *Phillips v. AWH Corp.*, 363 F.3d 1207, 1216 (Fed.Cir.2004). "It is only necessary and appropriate to file a cross-appeal when a party seeks to enlarge its own rights under the judgment or to lessen the rights of its adversary under the judgment." *Bailey v. Dart Container Corp. of Mich.*, 292 F.3d 1360, 1362 (Fed.Cir.2002). The conditional cross-appeal is dismissed as improper because the district court entered a judgment of noninfringement in Abbott's favor. *See Phillips*, 363 F.3d at 1216.

CONCLUSION

Although the district court's claim construction was erroneous, we find, under ***1340** our amended claim construction, no error in the district court's grant of the JMOL. We reverse the claim construction but affirm the grant of JMOL for noninfringement on claim 81 of the '840 patent.

AFFIRMED.

BRYSON, Circuit Judge, dissenting.

Claim 81 of the '840 patent recites a composition comprising four components: cyclosporin A; a propylene glycol hydrophilic component; a hydrophilic surfactant; and a lipophilic component. The lipophilic component is described in the patent as an oil-loving substance that dissolves the cyclosporin so that when water is added to the composition an oil-in-water microemulsion is created.

It is not disputed that the accused Abbott composition contains the first three of the claimed components; only the presence of a lipophilic component is in dispute. Novartis argues that the Abbott composition has a lipophilic component in the form of Span 80, a lipophilic surfactant. Abbott argues that although Span 80 is lipophilic, it cannot serve as the lipophilic component of claim 81. The court today agrees with Abbott, based on statements in the specification of the '840 patent. Although the court acknowledges that a lipophilic surfactant such as Span 80 can serve as *part* of the claimed lipophilic component, it holds that the specification does not allow for a surfactant to serve as the *entire* lipophilic component. Accordingly, the court holds that because Span 80 is the only lipophilic component in Abbott's composition, the Abbott composition does not infringe, either literally or under the doctrine of equivalents.

Because it is undisputed that Span 80 is lipophilic and that cyclosporin dissolves in Span 80, Span 80 appears to qualify as a "lipophilic component" as that term is used in the '840 patent. Thus, Abbott can prevail on this claim construction issue only if the specification is read as requiring that the "lipophilic component" limitation be construed more narrowly. The court today concludes that the specification has that effect, but I do not agree.

In reaching its conclusion as to the proper construction of the term "lipophilic component," the court relies on a passage from the specification stating that a surfactant may be incorporated in a microemulsion composition of the invention not only as the surfactant, "but in excess as an additional carrier or co-solvent phase, i.e. as part of the hydrophilic or lipophilic phase." '840 patent, col. 12, ll. 47-49. *See also id.*, col. 14, ll. 40- 42 (referring to a lipophilic surfactant serving "as a surfactant or as a co-solvent"); *id.*, col. 16, ll. 39-41 (referring to a surfactant that is "employed as a surfactant only or both a surfactant and a co-solvent"). The court interprets that language, which recognizes that a surfactant can serve as part of the hydrophilic or lipophilic components of the claimed composition, as meaning that a surfactant may be a part of the lipophilic component but may not serve as the lipophilic component by itself.

I do not draw the same conclusion from the quoted language. The statement on which the court relies refers to the role performed by excess amounts of the substance that serves the role of the surfactant in the

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claimed composition. The quoted language indicates that the composition of the invention must have at least three components in addition to the cyclosporin, as required by claim 81. In addition, it contemplates that the substance that serves as the surfactant may also serve as part of either the hydrophilic or the lipophilic component. The quoted language therefore makes clear that the composition may not consist of only two components ***1341** in addition to the cyclosporin, as would be the case if one ingredient served as both the surfactant and the lipophilic component in the composition. However, the quoted language does not address the case presented by Abbott's composition, which contains three components in addition to the cyclosporin, and in which a hydrophilic surfactant serves as the surfactant, while Span 80 serves the function of the lipophilic component (dissolving and carrying the cyclosporin). Thus, because in Abbott's composition Span 80 does not serve as the surfactant referred to in claim 81, the quoted language from the specification does not apply to Abbott's composition. For that reason, I agree with Novartis that, where another substance serves as the hydrophilic surfactant, the specification does not disclaim the use of a lipophilic surfactant such as Span 80 as the sole component of the lipophilic component of the claimed invention. I therefore do not find a disclaimer of subject matter in the specification. *A fortiori,* I do not find a specific exclusion of subject matter for purposes of the doctrine of equivalents.

I respectfully dissent.

375 F.3d 1328, 71 U.S.P.Q.2d 1650

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